

**PROCESS DEVELOPMENT AND PRODUCT
CHARACTERIZATION OF COWPEA FORTIFIED EXTRUDED
BREAKFAST CEREAL FROM LOW GRADE RICE**

BY

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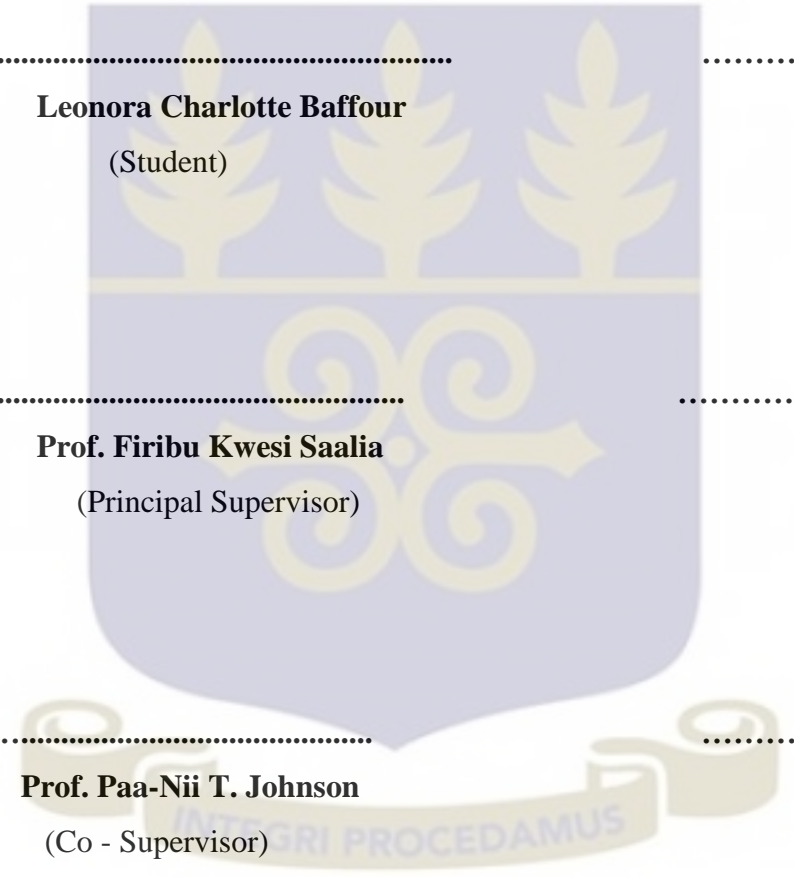
**THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA,
LEGON IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR
THE AWARD OF MPhil FOOD SCIENCE DEGREE.**

JUNE, 2015

DECLARATION

This is to certify that this thesis is the result of research undertaken by Leonora Charlotte Baffour towards the award of the MPhil Food Science in the Department of Nutrition and Food Science, University of Ghana.

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The background of the signature area features a large, light-colored watermark of the University of Ghana crest. The crest is a shield divided into two sections. The upper section contains three golden wheat stalks. The lower section contains a golden cross with four curved arms. Below the shield is a ribbon with the Latin motto 'INTEGRITATEM PROCEDAMUS'.

ABSTRACT

Rice production in Ghana is faced with the major challenge of low patronage. This is due to its poor physical and sensory quality rendering it 'low grade' rice. Among the many local varieties of rice in Ghana, *Viwonor* is perceived to be a low grade rice whereas *Jasmine 85* a high grade. Large quantities of rice are imported to address the issue of quality and quantity gap.

Protein energy malnutrition (PEM) is a serious nutritional problem facing many countries including Ghana. Cowpea (*Vigna unguiculata*) is an important legume rich in minerals and proteins. One of the most common and effective processes for improving the quality of legumes is sprouting. Addition of value to low grade rice which otherwise would be underutilized (in spite of its nutritional value) through the development of cowpea fortified extruded breakfast cereal will help in diversifying and improving the market potential of rice produced in Ghana. The main objective of this work was to study the characteristics of rice-sprouted cowpea extrudates and evaluate their performance and acceptability as a breakfast cereal.

Differences in rice cultivars significantly influenced ($p < 0.05$) its physical properties with lower % head rice being measured in *Viwonor* rice. Relatively higher apparent amylose, Gelatinization Temperature (GT) class and hardness index was measured in *Viwonor* rice. This is an indication of longer cooking quality and hence low quality rice. Protein contents and solubilities of cowpea significantly decreased ($p < 0.05$) with increasing sprouting time in all buffers. The SDS-PAGE results showed no significant variability in the number of protein bands for each germination day and by the different buffer solutions. Generally, cowpea proteins of higher molecular weight (45 and 66 KDa) were mostly extracted in all buffers. Cowpeas that were sprouted for three days were far more digestible by pepsin and pancreatin enzymes than those that were not sprouted for that

long. Increasing germination time of cowpea seeds significantly decreased ($p < 0.05$) the amount of accessible thiols with or without the denaturant.

Cowpeas were sprouted for 1, 2 and 3 days in addition to a control (unsprouted) and used to make breakfast cereal formulations with *Viwonor* rice flour (low grade) using the Central Composite Rotatable Design (CCRD). 13 flour formulations were generated and extruded using a twin screw extruder under the following conditions; barrel temperature = 200°C; screw speed = 1200 rpm; die diameter = 4.0 mm and moisture content = 25%. Consumer preference test was done on the extrudates. ANOVA was performed and the mean acceptability score was used to select the 3 most preferred extrudates. Extrudate 7 (22% 2 day sprouted cowpea + 78 *Viwonor* rice) was rated the most preferred extrudate; followed by extrudate 1 and 2 with 30% unsprouted cowpea + 70% *Viwonor* rice and 30% 3 day sprouted cowpea + 70 *Viwonor* rice. The corresponding flour formulation for the best 3 extrudates was used in extruding additional 3 extrudates from *Jasmine 85* rice (control). A total of 6 extrudates were obtained.

The SDS-PAGE results show that both rice and cowpea proteins contributed to the protein structure of the extrudates. Extrudates produced from unsprouted cowpea (Extrudates 1 and 4) showed the highest rate of hydrolyses by pepsin while the sprouted cowpea – based extrudates were low. Extrudates made from *Jasmine 85* rice were characterized with higher expansion ratios and bulk densities. Lower hardness values were measured among the *Jasmine 85* - based extrudates. Bubbles belonging to class 1 (0.01 – 0.1 mm²) and 2 (0.1 – 3 mm²) were dominantly present in all extrudates. The WS (sulphur containing) sensors of the Electronic Nose system separated the extrudates based on sprouted cowpea whereas the WC (arom-aliph compounds) sensors were responsible for discriminating the extrudates based on unsprouted cowpea.

With respect to the Electronic Tongue sensing system, *Viwonor* - based extrudates enriched with sprouted cowpea (extrudates 2 and 3) were characterized by sourness whereas *Jasmine* 85 - based extrudates with sprouted cowpea (extrudates 5 and 6) were characterized by astringency and bitter aftertaste. Extrudates made with unsprouted cowpea flours (extrudates 1 and 4) were perceived to be less bitter and astringent aftertaste.



DEDICATION

I dedicate this work to the Almighty God in whom I have my being. To my amazing parents, Mr. Robert Patrick Baffour and Madam Akosua Abrafi for sacrificing to bring me this far.



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All praise and glory be to the Almighty God by whose divine grace and strength has seen me through and more especially completing this work successfully. I wish to express my profound and inexhaustible gratitude to my supervisors Prof. F. K. Saalia and Prof. Paa-Nii T. Johnson for their immeasurable assistance and constructive criticism in which they guided and supervised my work. My prayer is that the Good Lord will recompense them for their assistance and perpetual good works.

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LIST OF ABBREVIATIONS

AOAC	Association of Official analytical Chemists
ASV	Alkaline Spreading Value
CIRAD	Centre International de Reseaux Agriculture and Development
FAO	Food and Agricultural Organization
GRIB	Ghana Rice Inter professional Body
GT	Gelatinization Temperature
ISO	International Standards Organization
JICA	Japan International Cooperation Agency
MAFAP	Monitoring African Food and Agricultural Policies
MOFA	Ministry of Food and Agriculture
PEM	Protein Energy Malnutrition
RMM	Rice Market Monitor
RTE	Ready to Eat
USAID	United States Agency for International Development
USDA	United States Development Agency
WARDA	West African Rice Development Agency
WHO	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Rice (*Oryza* spp.) is the most widely consumed staple providing about 20% of the world's dietary energy supply (FAO, 2006). The major rice growing countries in the world are China, India, Indonesia, Bangladesh, Viet Nam, Thailand, Philippines, Brazil and United States of America. The unique climatic condition (humid and hot) of the Asians gives them the advantage in leading the global rice production. In the African continent, West Africa leads in rice production in Sub-Saharan Africa (SSA) but it is faced with the challenge of meeting the demand as consumption rate outweighs that of production (West African Rice Development Agency WARDA, 2007).

In Ghana, the per capita consumption of rice increased from 17.5 kg per annum between 1999 and 2001 to 22.6 kg per annum between 2002 and 2004 (Amanor-Boadu, 2012). This shot up to 24 kg per annum by 2010/2011 (MOFA 2011a; Amanor-Boadu, 2012). This increase in per capita consumption of rice has placed it as the most important cereal crop after maize in Ghana (Amanor-Boadu, 2012). In spite of improvements of rice production trends in Ghana, significantly large quantities of rice are imported to address quality and quantity differences between local production and demand (Edache, 2005; Amanor-Boadu, 2012). Inadequate handling as well as poor harvesting, milling and transportation are major drawbacks in the production of quality rice locally. Gayin *et al.*, (2009) related the quality defects of the local rice to the high presence of organic and inorganic foreign materials, stones, weeds, seeds, with high levels of chalky grains, broken and damaged grains. In spite of the high cost of rice importation, the local rice suffers very low consumer acceptability with a consequent low market value. Indeed,

Marcela and Ashitey in 2011 reported that only 20% of the locally produced rice is consumed in the urban communities in Ghana, and these markets represent about 76% of the total rice consumed in Ghana. Poor planting materials and agronomic practices, late harvesting and inappropriate post-production handling practices are the result of the quality defects in the local rice (Manful *et al.*, 1998; Gayin *et al.*, 2009).

A great deal of research work has been done to study the quality characteristics of local rice varieties in Ghana, with the view to improving consumer acceptability. It has been established that the appearance of the rice grain, cooking quality as well as the taste are strong quality parameters consumers look out for in purchasing rice (Diako *et al.*, 2011). *Jasmine 85* and *Viwonor* cultivars are typical examples of local aromatic rice. The appearance of *Jasmine 85* aside its aromatic nature places it among the most preferred local cultivars of rice unlike the *Viwonor* which has a dark red coloration. *Viwonor* rice variety typically has high content of broken grains when milled, with a consequent reduction in quality grading. The *Viwonor* (brown rice) is classified under the *Glaberrima* cultivar of rice. The consumption and utilization of brown rice in value added food products has been on the increase due to their reputation for nutritional excellence and health claims (Heinemann *et al.*, 2005). Diako *et al.*, (2011) revealed that the local varieties of rice were nutritionally superior to the imported rice brands, as they generally contained higher iron (5.0-8.2 mg/kg) and phosphorous (1166-1374 mg/kg) contents. In spite of their poor acceptability due to appearance, incorporation of local rice varieties in processed foods, particularly breakfast cereals, might benefit from their high nutritional profile.

Breakfast is globally known to be the most important meal of the day. Studies have shown that individuals who consume breakfast like ready-to-eat (RTE) cereals, have better overall nutrition profiles, exhibit improvements in cognitive functioning, and are

less likely to be overweight (Rampersaud *et al.*, 2005 as cited by Schwartz *et al.*, 2008). Extruded breakfast cereal products notably rice flakes, corn flakes, wheat flakes and several other formulated breakfast cereal based product are common in the market and gaining rapid popularity. They usually come as ready-to-eat (RTE), and are consumed mostly in the urban communities particularly among school-going children and sedentary workers. The utilization of low grade rice could be improved by processing it into ready to eat breakfast cereal, in particular one with enhanced protein content to improve the nutritional status of consumers.

Protein energy malnutrition is one of the serious nutritional problems facing over 170 countries (Iqbal *et al.*, 2006; Anyango, *et al.*, 2011). Cowpea (*Vigna unguiculata*) is an important legume rich in minerals, protein and the essential amino acid lysine (Sharma *et al.*, 1999). The consumption of cowpea is high in Asia, Africa and South America (Frias, *et al.*, 2005) and nutritionally, cowpea has high-level of folic acid (Bressani, 1985).

Pre-process treatment procedures such as soaking, fermentation, sprouting and appertisation have been proven to reduce the presence of most antinutritional factors in legumes (Tabekhia and Luh, 1980; Marfo *et al.*, 1990; Honke *et al.*, 1998; Liu *et al.*, 2005; Afify *et al.*, 2011) including cowpeas. Germination/sprouting have been proposed as a useful and easy process for improving nutritional quality of legume seeds (Doblado, *et al.*, 2007). Germinated or sprouted seeds acquire the form, flavour, and consistency of a fresh food without loss of protein. One of the most common and effective processes for improving the quality of legumes is germination or sprouting (Lopez-Amoro *et al.*, 2006). Ready to eat breakfast cereals are made using a variety of technologies, but the most versatile, and most popularly used is extrusion cooking technology.

Extrusion cooking is a process in which food material is forced by the rotation action of Archimedean screw(s) along a tightly fitting heated barrel under high temperature, high-pressure, high shear and (relatively) low moisture to exit through a very narrow orifice die. As the food exits the die under high pressure, moisture is flashed off it and it spontaneously expands to provide a light and melt-in-the mouth pre-cooked product. Extrusion cooking is so versatile that it has created the possibility of obtaining a range of products that otherwise could not have easily been made with other methods of food processing. Extrusion cooking has been used in the production of protein fortified breakfast meals using legumes, pulses and nuts (Asare *et al.*, 2011; Mbaeyi and Onweluzo, 2010; Anyango *et al.*, 2011; Pastor-Cavada *et al.*, 2011).

1.2 Rationale

Low grade rice has low consumer acceptability with a consequent low market value in Ghana. The project aims at adding value to low grade rice which otherwise would be underutilized (in spite of its nutritional value) through the development of cowpea fortified extruded breakfast cereal. This will help in diversifying and improving the market potential of rice produced in Ghana. Formulating a breakfast cereal using low grade rice and sprouted cowpeas could provide an inexpensive but highly nutritious product.

1.3 Main Objective

The main objective of this work is to study the characteristics of rice-sprouted cowpea extrudates and evaluate their performance and acceptability as a breakfast cereal.

1.3.1 Specific Objectives

- To determine the physico-chemical properties of two cultivars of locally milled rice.

- To characterize the protein fractions of sprouted cowpea.
- To formulate rice - sprouted cowpea flour for the development of an extruded breakfast cereal.
- To study the quality characteristics of the extrudates.
- To study sensory attributes of the extrudates using Instrumental Sensory method.



CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Botany and Agronomy of Rice

Rice (*Oryza* spp.) is a monocot plant (*Oryza Sativa* or *Oryza Glaberrima*) belonging to the family of grass (*Poaceae Gramineae*) and originates from Asia or Africa. It is a semi aquatic annual plant and morphologically characterized by long sheathed leaves with hollow and erected stem. Generally, the growth duration of rice is from 70 to 160 days depending on the cultivar and environmental conditions (Luh, 1991). The two species of cultivated rice globally are the *Oryza Sativa* and *Oryza Glaberrima* (Linares, 2002).

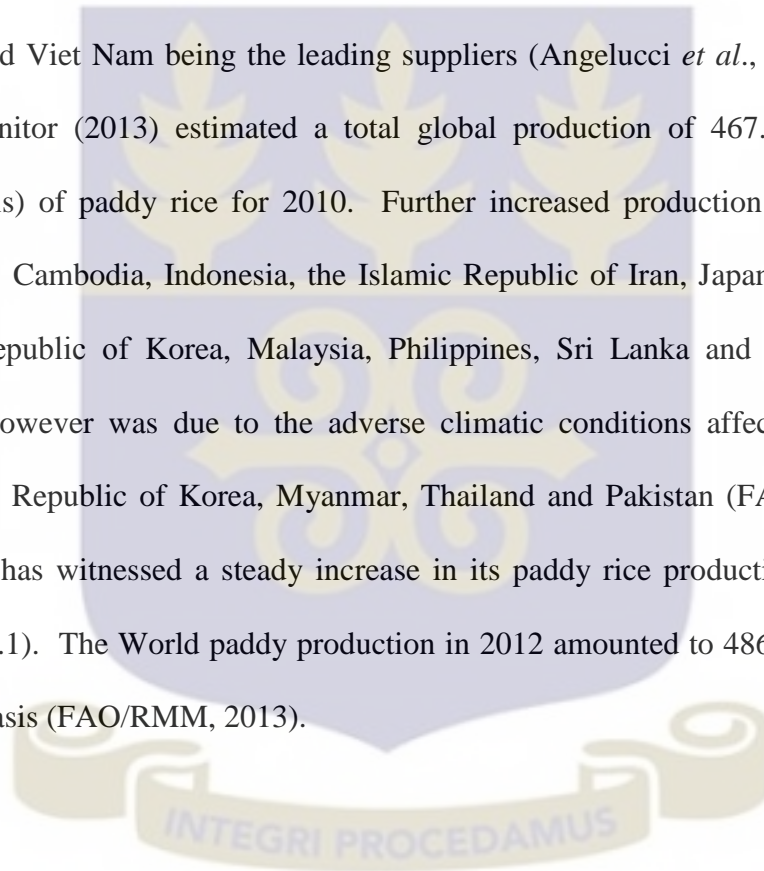
Three popular and broad sub-species (*indicas*, *japonicas* and *javanicas*) have been identified within the *Oryza Sativa* species (Linares, 2002; Nakamura *et al.*, 2002; Kennedy and Burlingame, 2003). The *indicas* and *japonicas* are the most common, with *indicas* representing 80% of the cultivated rice (Kennedy and Burlingame, 2003). These two cultivars are able to grow in a wide range of soil types from deep watered to dry and hilly lands (Luh, 1991). The *indica* varieties are typically common in tropical regions, as many are drought tolerant but do not tolerate colder temperatures (Kennedy and Burlingame, 2003). The *Oryza japonica* cultivars are generally referred to as ‘short or medium grain’ and are typically characterized by stickiness upon cooking and are due to the high presence of amylopectin while the *Oryza indica* are long grain and appear drier, flaky and remain separated upon cooking and usually low yielding (Luh and Mickus, 1991; FAO, 2000). *Japonica* varieties tend to have lower amylose content than the *indica* varieties (FAO, 2000).

It is estimated that about 20 different species of the *Oryza* genus have been recognized. However, nearly all cultivated rice is *Oryza Sativa* (Bienvenido, 1993). The *Oryza Sativa* has over the years developed a wide range of tolerance and adaptability due to its

history of cultivation and survival under diverse environmental condition (such as from flooded valley areas to dry hilly slopes). Comparatively, limited amounts of the *Oryza Glaberrima* cultivars are cultivated in some part of the African continent while the *Zizania Aquatic* (wild rice) which is morphologically closely related to oats are cultivated in the United States of America (Bienvenido, 1993).

2.2 Global Rice Production and Consumption

Between the period of 2005 to 2009, rice has been the most imported crop in Ghana with Thailand and Viet Nam being the leading suppliers (Angelucci *et al.*, 2013). The Rice Market Monitor (2013) estimated a total global production of 467.3 million tonnes (milled basis) of paddy rice for 2010. Further increased production was expected in Bangladesh, Cambodia, Indonesia, the Islamic Republic of Iran, Japan, the Democratic People's Republic of Korea, Malaysia, Philippines, Sri Lanka and Viet Nam. The drawback however was due to the adverse climatic conditions affecting nations like Taiwan, the Republic of Korea, Myanmar, Thailand and Pakistan (FAO/RMM, 2013). The World has witnessed a steady increase in its paddy rice production from 2003 to 2012 (Fig 2.1). The World paddy production in 2012 amounted to 486.9 million tonnes on milled basis (FAO/RMM, 2013).



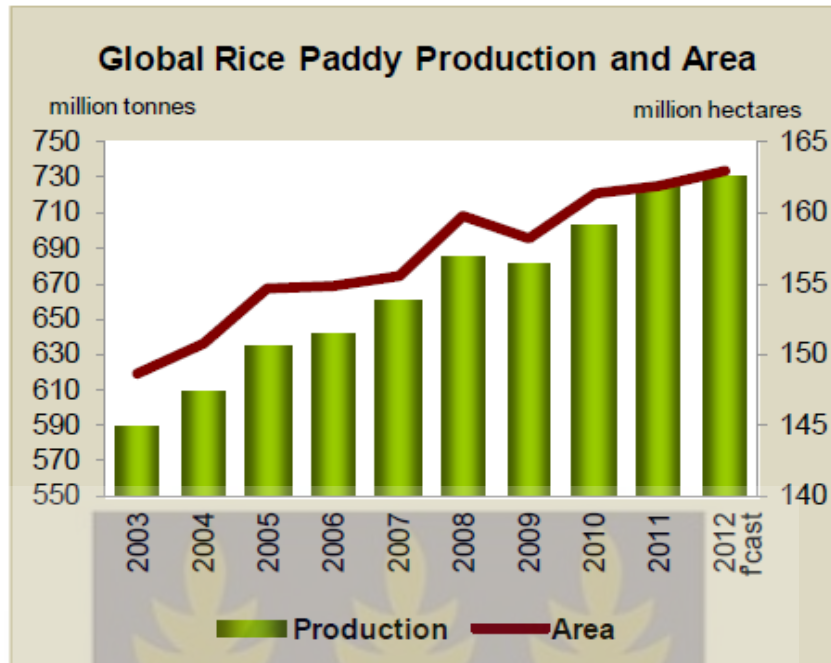


Fig 2. 1: Global Paddy Rice Production and Area

Source: Food and agriculture Organization/Rice Market Monitor (FAO/RMM), 2013.

2.3 Production and Consumption of Rice in Sub Saharan Africa (SSA)

The steady demand for rice over the past three decades has played a major role in the strategic food security planning policies of many countries. In most countries, consumption rate exceeds that of production and large quantities of rice continue to be imported to meet domestic demands at a high cost in foreign currency especially in Africa (Seck *et al*, 2010). Sub Saharan Africa (SSA) has resorted to the importation of larger volumes of rice to meet its growing consumption needs. Rice consumption and production increased at a rate of 4.5% and 3.2% per annum respectively between 1961 and 2006 in Africa (Seck *et al.*, 2010). Africa increased its rice importation from 5 million tonnes in the early 1990s to 12 million tonnes resulting in 140% in 2004. In 2007, a total amount of 25 million tonnes with an average per capita consumption of 24 kg per annum of milled rice was consumed in Africa (Seck *et al*, 2010). The total paddy rice production in Africa amounted to 23.4 million tonnes in 2007 (Seck *et al.*, 2010).

Among the many factors influencing the rising demand for rice within the SSA including per capita income, relative rice price and population growth, urbanization has been identified as the main driving force for rice consumption (Diagana *et al.*, 1999). Indeed, rice is no longer a luxury food in West Africa and it has become a major source of calories for the urban poor (Dingkuhn and Randolph, 1997). According to Seck *et al.*, (2010), the high prices of rice and consequent market instability has strong implications for global food security especially in areas where its consumption is rapidly increasing and also in areas where there is a high imbalance of the domestic production-consumption rate like the situation in the SSA. A report by United Nations UN, (2006) affirmed that the number of Africans living in urban areas is expected to increase from 38% to 48% (Seck *et al.*, 2010). This projected increase has the tendency of encouraging the growing trend of rice consumption in Africa. Seck *et al.* (2010) estimated that almost 40% of SSA's total rice consumption is imported. Lancon and Erenstein in 2002 forecasted that by the year 2020, total rice importation will be between 6.5 and 10.1 million tonnes.

2.4 Prospects of Rice Production in Ghana

Rice is the first imported cereal in Ghana accounting for 5% of the total agricultural imports of the country from 2005 to 2009 (CARD, 2010). Rice production in Ghana for 2010 was estimated at 247, 000 tonnes on milled basis representing a 5% increase over 2009 (FAO/RMM, 2013). The major drivers in the high growth of per capita consumption of rice in Ghana are urbanization, population growth and changes in consumer eating patterns. The fact remains clear that the consumption of rice in Ghana exceeds greatly its gross rice production annually (MoFA, 2009; Rondon and Ashitey, 2011). Aside the influence of population growth on the high consumption rate of rice in Ghana, rice can be conveniently prepared into variety of dishes (Rondon and Ashitey,

2011). This has placed rice in a lead position among the traditional staples in Ghana. Another key factor influencing the steady growth of rice consumption in Ghana is the rising numbers of operation of fast food vendors within the country.

The consumption of rice in Ghana in May 2011 totaled 620,000 million tonnes. Meanwhile the per capita consumption estimated for the year 2010/2011 was 25.83kg (Marcela and Ashitey, 2011). In developing countries, rice accounts for 715 kcal/capita/day 27% of dietary energy supply, 20% of dietary protein and 3% of dietary fat (Kennedy and Burlingame, 2003). Statistics from Ministry of Food and Agricultural (MoFA, 2009) revealed that average paddy rice production in Ghana from 1996 to 2005 was 200,000 to 280,000 tonnes (130,000 to 182,000 tonnes on milled basis). However, in 2005 alone, the country's total rice consumption amounted to 500,000 tonnes equivalent to per capita consumption of 22kg per annum (JICA, 2007 as cited by MoFA 2009). Owing to the above situation, Ghana relies strongly on the importation of rice to offset its deficit in annual rice supply.

2.4.1 Challenges in rice production in Ghana

The local rice production sector is faced with the major challenge of low patronage (Adu-Kwarteng *et al.*, 2003; Gayin *et al.*, 2009; 2003; Diako *et al.*, 2011). Other challenges include the land tenure system, socio-cultural issues, trans-boundary or regional issues, inadequate infrastructure among others (MoFA, 2009). It has been reported that greater parts of the populace in Ghana prefer imported rice to the locally produced rice mainly for quality reasons (Adu-Kwarteng *et al.*, 2003; Diako *et al.*, 2011). Gayin *et al.*, (2009) suggested that major factors accounting for variation in rice quality in Ghana are poor physical and sensory properties. The gap between domestic rice production and supply continues to widen as consumer demand for quality rice increases with a consequent change in consumer preference. Urban markets represent

about 76% of the total rice consumed in Ghana (MoFA Report 2009/2010); however only 20% of the locally produced rice is consumed in the urban communities in Ghana with the remaining being consumed in the rural areas (Marcela and Ashitey, 2011).

Rice is produced in 9 of the 10 regions of Ghana, the exception of Greater Accra region. However the bulk of local rice production (37%) is from the Northern Region while 27% and 15% are from Upper East and Volta regions respectively (Rgasa *et al.*, 2013). Several cultivars of local rice are cultivated and milled in Ghana. Some of them include the *Ex-Hoehoe Togo Marshall*, *Bouake*, *Ex-USA*, *Local perfumed*, *Jasmine 85*, *Ex-Baika*, *Viwonor*, *3108*, *ITA-304*, *B-189*, *Akpafu*, *GK-88*, *GK-49*, *IR-66*, *IR-64*, *IR-72*, *GRUG-7* and many more. While much of the locally produced rice is milled by polishing some of it is milled by parboiling. Indeed four new parboiled rice brands have recently been launched in Ghana and they include the Northern star, Gold star, Dada Ba and Oman Ba. A study by Diako *et al.*, (2011) classified *Jasmine 85* as a cultivar with high patronage. Another variety of locally grown rice that is performing well on the local market is the *Togo Marshal*. In an interview with officials from Ghana Rice Inter professional Body (GRIB), it emerged that many local cultivars of rice are gradually being lost due to low consumer patronage. Owing to this, Ghanaian farmers have resorted to cultivating only the few highly patronized cultivars to maximize income.

2.5 The Quality of Rice Grain

Grain quality essentially affects all stakeholders of the rice industry most especially consumers and processors. The quality and quantity losses continue to occur at the pre- and post-harvest phases, where many factors influence the ultimate quality of the grain (Velupillai and Pandey, 1990). The assessment of rice grain is based on physical and eating quality. The physical parameters defining the quality of rice include percentage

unbroken or whole kernel after milling whereas that of the eating qualities depend mainly on the starch composition (Manful, 2013).

Rice grains exhibit distinct physicochemical properties depending on their cultivars and the quality of starch largely influences the cooking properties of rice (Nakamura *et al.*, 2002). Diako *et al.*, (2011) reported that the consumer preference for rice is critically dependent on its appearance, taste and aroma as these attributes influence the final product. Tan *et al.*, (2000) suggested that appearance is determined mainly by the grain shape (specified by grain length, grain width, length-to-width ratio) and the translucency of the endosperm. The shape of the rice grain is controlled by triploid endosperm genes, cytoplasmic genes, embryo and maternal plant genes (Shi *et al.*, 2000). Generally, clear and translucent endosperm is required by many rice processing industries (Adu-Kwarteng *et al.*, 2003; Koutroubas *et al.*, 2004; Diako *et al.*, 2011). Chalkiness is another quality parameter of rice, and it occurs when rice is harvested at extremely high moisture level (Shi *et al.*, 1997).

Manful *et al.*, (1998) indicated that the quality of rice is often judged by the suitability for specific end use for the consumer. Rice quality studies by Diako *et al.*, 2011; Gayin *et al.*, 2009 and Manful *et al.*, 1998 suggested that majority of the local rice perform poorly on the market due to low quality. In spite of the fact that some local rice is naturally aromatic, the high content of foreign materials retards its patronage.

The problem of low patronage of locally processed rice is due to the high percentage of brokenness and presence of extraneous materials such as stones, rice husks, and other cereals or legumes. Gayin *et al.*, (2009) also outlined poor physical and sensory properties as the major factors undermining the quality of the locally produced rice. On the average, imported rice cost about 15-40% higher than locally produced rice and this

is mainly associated with good appearance (translucent and with long whole grains, less broken grains), taste, aroma and better quality (Ragasa *et al.*, 2013). Moreover, other quality characteristics of rice such as the texture and stickiness are important cooking and processing parameters that many rice processors look out for when evaluating rice (Koutroubas *et al.*, 2004).

Milling yield, grain size and shape and general appearance are important determinants of milling quality (Webb, 1985). The proportion of whole kernel and broken kernels produced during the milling of rice determines the milling yield; the remaining grains usually of three-quarters or more of the normal length is whole grain yield (Koutroubas *et al.*, 2004). Whole grain yield and broken grains constitutes total milling yield. Studies have shown that the breakage of grains during milling mostly depends on the variety, size and shape of the grain (Koutroubas *et al.*, 2004).

2.5.1 Physico-Chemical Parameters Defining the Quality of Rice Grain

Quality of rice is defined and measured by its physico-chemical and sensory parameters (Shinde *et al.*, 2014). And physical and sensory qualities are the most predominant factors affecting variability in rice cultivars (Tomlins *et al.*, 2005). Diverse criteria for judging the quality of rice are set by several users depending on the desired end product and consumer preference. Generally, rice quality test are essentially based on grain quality indices and consumer preference (Diako *et al.*, 2011). Gayin *et al.*, (2009) noted that the physical and sensory qualities accounts for the quality of rice grain and appearance, milling quality, cooking, processing and nutritional quality are the most important quality assessment parameters of rice (Koutroubas *et al.*, 2004).

Rice grain quality attributes include physical (milling quality, hardness, grain length and shape) and biochemical (amylose content, gel consistence, gelatinization temperature and aroma) characteristics. Grain length, shape and weight are objective means of

categorizing rice into three classes (Diako *et al.*, 2011). Grain length is defined as a measure of the longest dimension of the grain. The shape of the grain is estimated by the ratio of the length and width of the grain. Rice grain length or size can be categorized into 4 classes which include: Extra-long (above 7.5 mm), Long (6.6 to 7.5 mm), Medium (5.5 to 6.6 mm) and Short (5.5 mm or less) (IRRI, 2002).

The local and international markets for rice revolves vitally around the physical quality indices of the rice grain and typical of such include the size, shape and grain uniformity (Webb, 1985).

Grain chalkiness can be broadly described as the presence of whiteness usually on the edges or central part of the milled rice (Manful, 2013). Chalkiness in grains occurs during the early stage of grain development due to adverse climatic conditions such as high temperature during grain filling (Shi *et al.*, 1997). Tashiro and Wardlaw (1991) and Lisle *et al.*, (2000) also reported that high temperatures during grain development tend to increase the occurrence of chalky grains in rice. High temperature at the time of grain filling results in loosely-packed starch granules and higher chalk occurrence in rice grains (Tashiro and Wardlaw, 1991). Rice grain can be said to be chalky if part or the entire milled rice is opaque. Chalkiness in rice disappears upon cooking and although it rarely affects the rice, it has negative influence on the quality of milled rice (IRRIb). Singh *et al.*, (2003) reported that chalkiness in grain reduces resistance to forces during milling process and results in decreased head rice recovery.

Several studies have demonstrated the physico-chemical and structural properties of rice starch (Varavinit *et al.*, 2003; Vandeputte *et al.*, 2003; Singh *et al.*, 2003; Vandeputte and Delcour, 2004; Sasaki *et al.*, 2009; Majzoobi1 and Farahnaky, 2009; Detchewa *et al.*, 2012). A study conducted by Singh *et al.*, (2003) on the physico-chemical,

morphological, thermal, cooking and textural properties of chalky and translucent rice kernels indicated that lower kernel hardness and amylose content were measured in chalky kernels compared to translucent kernels. Results of a study of the differences in cooking and eating properties between chalky and translucent rice grains by Cheng *et al.*, (2005) showed that the chalky rice recorded dramatically high transition temperatures and hardness than the translucent rice.

Another important rice quality parameter is amylose content. Amylose is slowly digested and provides beneficial effects on human health (Hu *et al.*, 2010). Research has shown that amylose has significant impact on the texture and cooking time of rice. Amylose content is important for the determination of cooking and pasting behaviour of rice (Adu-Kwarteng *et al.*, 2003). Amylose in itself has the tendency of restraining the swelling capacity of starch granules with consequent adverse effect on the peak viscosity of the rice starch (Noosak *et al.*, 2003). It is essential to note that higher environmental temperature has been shown to decrease amylose content in endosperm of non-waxy rice (Asaoka *et al.*, 1985).

Gelatinization temperature is the temperature at which rice absorbs water and the inherent starch begins to irreversibly swell (Shinde *et al.*, 2014). Rice cultivar with high gelatinization temperatures are resistant to water uptake and gelatinization since high gelatinization temperatures is an indication of a crystalline structure (Metcalf and Lund, 1985).

2.5.2 Physical Form and Composition of Rice Starch

Starch forms the major component of the rice grain and is commonly used in the food industry. Milled rice consists primarily of about 90 % starch hence the physicochemical and structural properties of rice starch play a key role in the choice of rice cultivars for

many industrial applications (Noda *et al.*, 2003). Rice starch has unique properties such as bland taste, white colour, easy digestion and non allergenicity rendering it useful in both food and pharmaceutical industries (Jirapa *et al.*, 2001; Shih, 2004; Hagenimana *et al.*, 2006; Yano, 2010). The relative low levels of lipid and proteins in rice accounts for its bland taste. The textural properties of rice flours are mainly influenced by starch due to its gelatinization, retrogradation and rheological behaviour (Lin *et al.*, 2011). Gelatinized rice starch has bland taste and is smooth, creamy and spreadable making it a good custard starch (Singh *et al.*, 2006).

Grain starch quality attributes vary among varieties and production environments (Unnevehr *et al.*, 1992; Fan *et al.*, 1999). Processed native starches are used widely in non-food applications such as the production of cosmetic dusting powder, photographic paper powder, laundry stiffening agent, paper, excipient for pharmaceutical tablets as well as for sugar coating in confectionaries (Singh *et al.*, 2006).

Singh *et al.*, (2003) reported that the properties of starch depend on the physical and chemical characteristics notably granule size, granule size distribution, amylose/amylopectin ratio and mineral content. Rice cultivars have great diversity in their composition, granule size, gelatinization, textural and cooking properties (Singh *et al.*, 2005). The granule of rice starch has been found to be very minute giving it a smooth texturing property similar to that of fat (Champagne, 1996). Generally all starches including rice starches are composed of amylose and amylopectin. Amylose is a linear molecule containing α - (1-4) –linked D-glucopyranosyl units while amylopectin has short α - (1-4) –linked D-glucosyl chains with 5-6% non-randomly distributed α - (1-6) –linked D-glucosyl bonds (Yasui *et al.*, 1996; Vandeputte *et al.*, 2003; Hu *et al.*, 2010). Physicochemical properties such as amylose content, swelling power and water

binding capacity have been significantly correlated with average granule size of starches from diverse plant sources (Singh and Singh, 2001).

Milled rice contains about 90% starch hence its structure and physico-chemical properties are the main features in rice cultivar selection for specific industrial purposes (Noda *et al.*, 2003). The starch granule structure is described in terms of amorphous and semi-crystalline rings, and the semi-crystalline natures of starch are ascribed to double helices formed by amylopectin branches (Vanderputte *et al.*, 2003).

The amylose content is a major determinate of the stickiness of rice. Rice with high amylose content is sticky; as the amylose content decreases, the rice becomes flaky or firmer (FAO, 2000). Amylose is smaller in terms of molecular weight compared to amylopectin and is largely linear in configuration. The number of chains of amylose molecules and the degree of polymerization depends on the plant source (Ottenhof and Farhat, 2004). Studies by Takeda *et al.*, (1987) reported on the incomplete hydrolysis of the amylose fraction by β - amylase and concluded that amylose may contain some α (1 \rightarrow 6) D-glucopyranosyl unit making the molecule slightly branched. Amylose content varies among rice cultivars from zero in waxy rice to 30% in non-waxy rice or normal genotypes (Vanderputte *et al.*, 2003b).

The degree of polymerization and chain length of amylose in rice ranges from 987 to 1225 and from 276 to 430 glucose units respectively (Aboubacar *et al.*, 2006). On the other hand, amylopectin from rice has an average degree of polymerization and chain length ranging from 2700 to 1000 and from 128 to 586 respectively (Aboubacar *et al.*, 2006); and these variations in rice starch are basically due to growing environment and genetics (Wickramasinghe and Noda, 2008; Copeland *et al.*, 2009).

Starch granules when heated in excess water swells up and gelatinizes. The molecular order of the starch granule is destroyed and the starch becomes easy to digest (Sasaki *et al.*, 2009). Upon heating, starch retrogradation occurs resulting in gel formation (Karim *et al.*, 2000; Sasaki *et al.*, 2009). The structure of starch granule is defined in terms of amorphous and semi-crystalline growth rings. The amorphous rings contain amylose and sometimes less ordered amylopectin (Morrison, 1995). A typical A-pattern was observed when the crystalline structure of rice was studied using X- ray diffraction technique (Majzoobi and Farahnaky 2009).

Extruded starchy foods undergo gelatinization and destruction of crystalline structure and molecular fragmentation of starch-lipid and protein- lipid complexes (Ho and Izzo, 1992). The process during which intermolecular bonds within the starch granules are broken down in the presence of heat and water is called starch gelatinization. This phenomenon promotes the interaction of the hydrogen bonding sites of the starch granules with water, resulting in irreversibly dissolved and highly swollen starch granules (Karim *et al.*, 2000; Sasaki *et al.*, 2009).

Starch gelatinization involves granule swelling, pasting, loss of birefringence and unravelling and melting of double helices (Singh *et al.*, 2012). Granule swelling, melting of crystals or double helices and leaching of amylose essentially take place during starch gelatinization. High peak viscosities are generally associated with higher swelling capacity of the starch granules. Different rice cultivars differ in swelling power and solubility. Starch gelatinization temperature generally depends on: the source; the amount of water in the system; types and concentration of salts, sugars, fats and proteins in the recipe; pH; and the employed technology. Vanderputte *et al.*, (2003) indicated that the high presences of long amylopectin chains are known to increase gelatinization temperatures. Cooking properties of starch and gel behaviour are reflected in the

pasting properties (Majzoobi and Farahnaky 2009). Pasting properties are also affected by the chain length of amylose and amylopectin as well as environmental and post-harvest treatments (Srichuwong *et al.*, 2005).

Pasting property of rice starch is directly correlated with amylose content (Han and Hamaker, 2001). Starch retrogradation is of great interest to many food scientist and technologist as it affects the quality, acceptability and shelf-life of starch-containing foods (Zhang *et al.*, 2013).

Granules are the physical form of small aggregates of starch polymers in cereals. Portions of starch polymers are crystalline regions and these crystalline regions exist in granule structures that hold the bulk of the polymer in a firm and rigid form. They also rotate the plane of polarized light introducing birefringence. Rice kernels possess a cluster of small globular granules of about 1-10 μ m.

2.5.3 Rice milling and nutritional quality

Milling quality consists of the total amount of milled rice obtained after milling (total milled rice yield) and the remaining part which is whole kernels thus head rice yield (Shinde *et al.*, 2014). High head rice after milling is indicative of high market value (Alizadeh, 2011). Brown rice can be further processed into different products once the paddy rice is milled. Milling of paddy rice can be done in three forms. Direct milling of the paddy into brown rice (undecorticated), further milling of brown rice into white or polished rice and the third category is parboiling of the paddy prior to milling (parboiled rice). Abrasion milling of rice is a common means of obtaining polished rice. Milling affects the presence of phytic acid and other nutrients due to their non-uniform distribution in the brown rice (Wang *et al.*, 2011). Rice is consumed in three traditional forms; white, parboiled and brown (González *et al.*, 2013). Parboiled and brown rice have low consumer acceptability due to their darker colour compared white polished

rice. Parboiling of rice is a process where the paddy is soaked in hot water or steamed before drying and milling. Parboiled rice is known to be superior in terms of nutrients as it retains more of the grain's inherent minerals and vitamins (notably vitamin B1) as compared with the polished or white rice.

2.6 Nutritional Profile of Rice

Rice is an important source of energy, vitamins, minerals, amino acids and other nutrients to about half of the world's population (Shih, 2004). Unpolished rice contains approximately 7.3% protein, 0.8% fibre, 64.3% available carbohydrate, 2.2% fat and 1.4% ash content (Zhou *et al.*, 2002). Unmilled rice is rich in dietary fibre (Shinde *et al.*, 2014). Milled rice has relatively low nutritional profile as processing methods greatly influence the amount of nutrients retained in the end product. Studies by Mensa-Wilmot *et al.*, 2001, Nnam, 2001; reported that most cereals are deficient in some essential nutrients such as threonine and tryptophan. Rice is deficient in lysine and is rich in glutamic and aspartic acid (Kanu *et al.*, 2009; Shinde *et al.*, 2014).

Proteins in cereals are generally categorized into albumin, globulin, prolamin and glutelin according to their solubility and prolamin and glutelin are the two major storage proteins of rice grains (Ning *et al.*, 2010). Kennedy and Burlingame (2003) reported that proteins are the second most abundant component of rice grain occupying about 9% of its dry weight. However, compared to other cereals, rice protein is relatively lower but has higher protein quality due to its higher prolamin/glutenin ratio (Kaul and Raghaviah, 1975). Rice protein is deficient in the essential amino acid lysine rendering it poor nutritionally.

Protein in brown rice is more concentrated in aleurone layer, the embryo and the subaleurone layer of the endosperm compared to the deeper starchy endosperm (Salinas-Moreno *et al.*, 1999). Prolamin is generally concentrated in the outer layers while

glutelin concentrated towards the centre of the endosperm (Leesawatwong *et al.*, 2005). Essentially, protein in rice has been suggested to be a major factor in determining the texture, pasting and sensory properties of milled rice (Ning *et al.*, 2010). There is enough evidence from numerous studies that proteins play vital role in determining the functional properties of the starch such as inhibiting the swelling of starch granules, reducing the pasting and crystallization capacities and increasing the pasting temperature of the isolated rice starch (Shih, 2004).

2.7 Production of Cowpea

Cowpea, (*Vigna unguiculata*) also known commonly as black-eyed pea is an essential legume in tropical Africa (Singh *et al.*, 1997) and it belongs to the Leguminosae (Fabaceae) family. Legumes like cowpea are sometimes referred to as grain legumes because they are usually grown for their edible seeds (Iqbal *et al.*, 2006). Majority of the cowpea cultivated worldwide (about three-fourths of the area of production) is located around the Sudan Savanna zones of the Sub-Saharan African belt. Notable countries include Senegal, Nigeria, Niger, Sudan, Kenya, Tanzania, Angola, Botswana and Mozambique. Other countries in South America (mostly Brazil), Asia, and the southeastern and southwestern regions of North America also produce cowpea (Ehlers and Hall, 1997). The world production of cowpea in 2003 amounted to 3722 thousand metric tonnes (FAO, 2004).

2.7.1 Economic Importance of Cowpea

Nnanna *et al.*, (1988) described cowpea as relatively inexpensive source of legume protein compared to many animal protein products. It is of much economic importance to many developing countries especially within the Sub Saharan African continent. Prinyawiwatkul *et al.*, (1996) also confirmed that cowpea is of good economic value and a promising food ingredient. Cowpea provides about 80% of dietary proteins in Nigeria

(Uwaegbute *et al.*, 2000). In Ghana, cowpea is consumed as boiled or steamed, it can also be eaten together with rice or gari (roasted and fermented cassava grits). It also plays significant role in the preparation of sauces, soups, snacks, weaning, complementary and breakfast based porridges. Chinma *et al.*, (2008) also emphasized the importance of cowpea as a grain legume for, vegetable and fodder for animals.

2.7.2 Nutritional Profile of Cowpea

Grain legumes are essential in human nutrition since they are rich source of proteins, carbohydrates, dietary fiber, B-complex vitamins (B1, thiamin and niacin), minerals (Ca, Mg, Zn, K, Fe and P) and essential amino acids (Taiwo, 1998). Grain legumes additionally contain health promoting phytochemicals such as phytosterol which has been recently associated with the prevention of cancer (Tiwari and Singh, 2012). Additionally, studies by Kahlon *et al.*, (2005) has revealed that the high consumption of legumes among the Asian community have resulted in the incidence of relatively low blood circulation related diseases.

Cowpeas are essential food sources and have significantly contributed to human nutrition globally. Cowpea flour generally is good for food fortification because it is highly nutritious. Ravindran *et al.*, (2011) reported that peas are protein-rich with low glycaemic index and are effective component for formulating foods. They are highly concentrated in proteins, carbohydrates and dietary fiber and play vital contribution to human nutrition in many countries (Lopez-Amoro *et al.*, 2006). They may exhibit some unique properties depending on the cultivar and the processing technique employed.

Depending on the environmental and genetic factors, nutritional and chemical compositions of cowpea, as well as its cooking properties vary considerably (Avanza *et al.*, 2013). Cowpea is known to be rich in lysine, approximately 486 mg/g nitrogen

making it an excellent protein quality enhancer when used in fortifying other cereal grains which are lysine deficient but rich in sulphur amino acids like methionine and cysteine (Prinyawiwatkul *et al.*, 1996).

Averagely, cowpea contains about 23-25% protein and 50-67% starch (Nielsen *et al.*, 1993; Phillips *et al.*, 2003) as cited by Yeung *et al.*, 2009. Cowpea contains 24–26% crude protein and is high in lysine, aspartic acid and glutamic acid but low in sulphur containing amino acids (Prinyawiwatkul, *et al.*, 1996). It has similar dietary profile as oats bran but relatively cheaper (Taiwo, 1998). Consumption of legumes generally has numerous health benefits especially in preventing metabolic related illnesses such as diabetes mellitus, coronary heart disease and colon cancer (Simpson *et al.*, 1981 as cited by Mune Muneb *et al.*, (2013). The health benefits of legumes are associated with the high contents of bioactive compounds with antioxidant properties. The presence of high proteins (20-50%) and complex carbohydrates which are mostly dietary fibre and water soluble polysaccharides renders legumes low glycemic potency (Ravindran *et al.*, 2011).

Several studies on cowpea have proven that it contains some amount of antinutritional factors rendering it undesirable in many instances. The presence of indigestible oligosaccharides (raffinose and stachyose) in legumes can induce flatulence and hence impede its usage in food applications (Omueti and Morton, 1996; Uwaegbute *et al.*, 2000). Studies by Borejszo and Khan, (1992) have shown that the inherent oligosaccharides can be drastically reduced by pre-processing treatments such as germination. According to Weaver and Kannan, (2002), phytates are strong chelating agents that function by suppressing the bioavailability of bivalent cations. Yeung (2009) also observed that the long cooking time of some cowpea cultivars is undesirable owing to the relatively high cost of energy required to soften grains.

2.7.3 Effect of Sprouting on the Nutritional Properties of Cowpea

One of the most common and effective processes for improving the quality of legumes is germination or sprouting (Jirapa *et al.*, 2001; Lopez-Amoro *et al.*, 2006). Sprouted legumes are widely consumed around the globe. There is rapid growth of interest in the use of sprouting as pre-process treatment of legumes partly due to the low cost energy used. Kuo *et al.*, (2004) indicated that germination of legume seeds for human consumption has gained much popularity in recent times partly because it is a simple and effective processing method for achieving desirable changes in food products as well as the achieving nutritional quality.

The use of sprouts as food has been in existence for many generations; however, the selection of seeds for consumers has been dynamic and rapidly increased in recent years Kuo *et al.*, (2004). Sprouting is a process where dormant but viable seeds are induced to start growing into seedling (Mbaeyi and Onweluzo, 2010). Sprouted legumes are consumed directly or dehulled, roasted and ground for further use. Sprouted seeds are known to acquire the form, flavour and consistency of a fresh food. Flavour quality of food products are enhanced during germination (Torres *et al.*, 2007; Enwere, 1998). Additionally, nutritional profile and digestibility of the food product is increased while antinutritional factors are reduced. The bioactive compounds resulting from the germination process contains some increased levels of antioxidants which is beneficial to the consumer. The protein and vitamin contents are also maintained (Gallup and Reder, 1943). Proteins and starches are hydrolyzed during fermentation; additionally, the quality and digestibility of the food is enhanced (Obatolu *et al.*, 2000).

Cowpeas, as legumes have high content of proteins. The protein content of cowpea has been reported to range from (23-25%). Most of these proteins have reported to be salt soluble globulins containing disulphide linked pairs and albumins with other

polypeptides (Fotso *et al.*, 1994). Ghavidel and Prakash, (2007) reported that germination improved protein digestibility of legumes.

During sprouting, certain enzymes that partially hydrolyzes the proteins, starches and oligosaccharides are activated. Sprouting suppresses the presence and potency of some antinutritional factors like tannins, phytates, trypsin inhibitors and many more. The enzymes (alpha-and beta amylases) convert carbohydrates into soluble sugars such as maltose and glucose and these sugars are utilized by the germinating seeds (Mbaeyi and Onweluzo, 2010). A study conducted by Gallup and Reder, (1943) revealed that the dry matter of the sprouted seeds was higher than that of the original seeds in all constituents except nitrogen-free extract. The high protein content of the cowpea and mung bean sprouts, are nutritionally important. Gallup and Reder, (1943); Henry and Massey, (2001) reported that B-complex vitamins (riboflavin, niacin, biotin and pantothenic acid), vitamin C and mineral content increase significantly during sprouting of cereal and legume grains.

The high levels of antinutritional factors such as trypsin inhibitors, phytates, tannins and oxalates in cereals and legumes have been shown to be reduced through sprouting (Mbaeyi and Onweluzo, 2010) and other thermal processing techniques (Lestienne *et al.*, 2005). Majority of antinutritional factors are drastically inactivated through appropriate heating and food processing techniques (Rangel *et al.*, 2004). The phytic acid present in some cereals and legumes binds to the inherent mineral acid and reduces the mineral bioavailability. The decrease in phytic acid content in sprouted legumes and cereals is due to increase activity of endogenous phytase during germination (Afify *et al.*, 2011 as cited by Wei *et al.*, 2013). Liu *et al.*, (2005) reported that phytic acid usually constitute about 1-5% of many cereals (on dry matter basis).

2.8 Production of Breakfast Cereals

Breakfast cereals are usually grouped into two; traditional (hot) cereals and ready-to-eat (cold) cereals. The former requires cooking or heating prior to consumption while the latter can be consumed instantly by the addition of sugar and milk. The traditional breakfast cereals are made using oats, farina (wheat), rye, rice, and corn. Majority of the traditional breakfast cereal on the market are produced from oats and wheat. The remaining cereals are made from rice, corn (excluding corn grits). Ready-to-eat cereals can further be classified by the processing technique employed. They include; flaked cereals, extruded flaked cereals, gun-puffed whole grains, extruded gun-puffed cereals, oven-puffed cereals, shredded whole grains, extruded shredded cereals, and granola cereals. Ready to Eat (RTE) breakfast cereals are described as highly expanded extruded products produced from cereal flour and starch ingredients (Clerici, 2012).

2.8.1 Cereal –Legume Complementation

The concept of cereal legume complementation has been widely applied in the development of infant complementary foods with the aim of increasing the protein content since the high protein content of the legume will significantly impact on the overall protein of the food. Cereals are known to be generally limited in amino acids. These cereals are basically available worldwide and in places where they are not cultivated, they are made available through importation. Research on the use of legume-cereal in various weaning /complementary foods and RTE breakfast cereals has been reported by Ejigui *et al.*, 2007, Mensa-Wilmot *et al.*, 2001; Kanu *et al.*, 2009; and Singh *et al.*, 2012).

2.8.2 Importance of Breakfast Consumption

Several health and nutritional benefits are derived from the consumption of breakfast. Studies by Wesnes *et al.*, 2003 also supported the assertion that breakfast consumption

improves cognition and learning function among children (Barton *et al.*, 2005). Reeves *et al.*, (2013) also reported on the correlation between the type of breakfast consumed and its impact on cognitive function. Individuals who consume cereals have improved nutrient status and health benefits (Albertson *et al.*, 2008). Kent and Worsley (2010) also reported that regular consumption of breakfast has been one of the strategies used by successful long-term weight loss maintainers in the National Weight Control Registry. Studies by Albertson *et al.*, 2008; Rampersaud *et al.*, 2005; Affenito, 2007; Panagiotakos, *et al.*, 2008 have highlighted the benefits of breakfast consumption most especially positive effect on dietary health and increased academic performance associated with the positive effect on cognition function of the consumer. In spite of the numerous health benefits of breakfast, Hill, (1995) reported that many dietary deficiencies are the result of breakfast omission.

Breakfast consumption has usually been associated with intake of some essential nutrient and quality diet among children and adolescent especially (Affenito, 2007). A study by Albertson *et al.*, (2008) discovered that the more cereal consumed, the better the macronutrient profile. Story and French in 2004 reported that breakfast consuming children have a higher tendency of meeting recommended intakes for vitamins and minerals than those who skip breakfast (Barton *et al.*, 2005).

Regular consumption of breakfast can prevent the onset of obesity among adolescents (Affenito *et al.*, 2005 as cited by Panagiotakos *et al.*, 2008). According to Burke (2006), the increased occurrence of some lifestyle diseases notably Type II diabetes (mellitus) and cardiovascular diseases among many young people have been shown to be associated with regular consumption of breakfast (Panagiotakos, *et al.*, 2008). Studies by Djousse and Gaziano, 2007; Van der Heijden *et al.*, 2007 concluded that breakfast

cereals are an important meal for the masses and has myriad of beneficial impact on various cardio-metabolic disorders like diabetes (DM) obesity and heart failure.

2.9 Extrusion Cooking

Cereal food manufactures have responded to the advantage of developing new whole grains and fibre-rich products, mostly in the form of flakes or blends (González *et al.*, 2013). Mariotti *et al.*, (2006) reported that the most used processes for the production of precooked cereal foods are flaking, puffing, and extrusion. Extrusion is a cost effective and an efficient industrial processing method which has enjoyed worldwide acceptance (Llo *et al.*, 2000; Ravindran *et al.*, 2011; Thachil *et al.*, 2014). Extrusion process is one of the most used process technologies for the production of snack foods, due to their technological benefit over the other methods (Włodarczyk-Stasiak and Jamroz, 2008; Caltinoglu *et al.*, 2013).

Extrusion process combines high temperature and pressure with mostly high shear (Ravindran *et al.*, 2011). The technology presents a myriad of advantages over the other processing techniques especially in the preparation of ready-to-eat (RTE) foods of desired size, shape, texture and sensory characteristics at low cost (Llo *et al.*, 2000; Sumathi *et al.*, 2007). In recent times, extrusion cooking has become an ideal method for manufacturing diverse food products including snacks, breakfast cereals and baby foods; it permits the production of increased proteins and starch digestibility and nutritionally balanced foods at a lower cost (Singh *et al.*, 2007).

Extrusion cooking of food is a process in which a food material is forced to flow under one or more conditions of mixing, heating and shear, through a die which is designed to form and/or puff-dry the ingredients. The effectiveness of the process and suitability of ingredients are often reflected by the physical characteristics of the extrudates. Extrusion as a unit operation is highly versatile and can be applied to a variety of food processes;

and results in products of unique physical and chemical characteristics (Pansawat *et al.*, 2008). It plays an important role in the food industry as efficient and convenient manufacturing processes. The rotating screw exerts shear energy in addition to the heated barrel, the food material is heated to its melting point or plasticating point and the food is conveyed under high pressure through a die. The resultant product usually expands and undergoes series of chemical and physical transformation (Moscicki and Van Zuilichem, 2011).

Extrusion cooking can be done at relatively low temperature (in pasta processing) or at high temperatures (extruded cereals and snacks). The use of extruders in food processing can be continually productive with high turnover. The application of high temperature short time (HTST) cooking retains many heat sensitive components of a food while eliminating microbial load of the product. Extrusion cooking is considered not only a High Temperature Short Time (HTST) process, but also a versatile one to obtain a wide range of ready-to-eat cereal foods (González *et al.*, 2013). The use of the High Temperature Short Time is advantageous to vulnerable food as the short time restricts unwanted denaturation effects on proteins, amino acids, vitamins, starches and enzymes (Moscicki and Van Zuilichem, 2011).

Extrusion is an environmentally friendly procedure which does not produce significant process effluents resulting in reduced costs of water treatment and levels of environmental pollution. Extrusion cooking technique has been extensively applied in the restructuring of starch and protein-based materials to manufacture a variety of texture convenience foods (Mbaeyi and Onweluzo, 2010). The technology has undergone a series of evolution from simple conveying device to advanced sophisticated processing as mixing, shearing, separation, heating or cooling, co-extrusion, venting volatiles and

moisture, flavour generation, encapsulating and sterilization in addition to conveying (Guy, 2001).

2.9.1 Types of Extruders in the Food Industry

Three types of extruders present in the food industry include screw-type extruders, roller and hydraulic ram (piston). They consist mainly of the screw, barrel, die, feed system, and drive systems. The screw extruder specially has a continuous processing and mixing ability that distinguishes it from the other two types of extruders. The dry extruders, interrupted-flight screw extruders, single and twin - screw extruders are typical examples of the screw extruders. The most widely used extruders are the single and twin-screw extruders (Ahmed, 2012). The Twin-screw food extruders are more complex and more universal in terms of design. Twin-screw extruders have gained popularity in food industries because of their high versatility (ability to process myriad of materials), lower energy consumption and the ability to increase the production (Moscicki and Van Zuilichem, 2011). The co-rotating twin-screw extruders are mostly used for the production of RTE breakfast cereals and are characterized by good efficiency of material transportation, mixing, plasticizing and extrusion. It also possesses the self-wiping ability (Moscicki and Van Zuilichem, 2011).

2.9.2 Principles of Extrusion Cooking

Several functions such as agglomeration, degassing, dehydration, expansion, gelatinization, grinding, homogenization, mixing, pasteurization and sterilization, protein denaturation, texture alteration, thermal cooking, shearing, shaping, and unitizing may occur during extrusion depending on the ingredients, extruder settings and the end product. Two important variables influencing the processing window during manufacturing are the machine variables and raw material characteristics. Product success depends on the optimization of these variables, and thus an optimization of these

variables results in quality product with strong sensory and nutritional characteristics (Guy, 2001). Usually, moisture levels of 10 to 40% on wet basis are used in extrusion. The application of high temperature reduces the cooking time and transforms raw materials fully within short duration from 30 to 120 s (Guy, 2001).

Extruded products differ from conventional foods in that their basic protein-fat and carbohydrate units may be derived from many sources which are combined with flavours and colours to form refined and desired products (Mbaeyi and Onweluzo, 2010). The native organized structure of the macro structure molecules within the food ingredients is lost during extrusion cooking and continuous dough is formed (Enwere, 1998). Mbaeyi and Onweluzo, (2010) reported that “the laminar flow within the channel on the extrusion screw and the die aligns the large molecules in the direction of flow, exposing bonding sites which lead to cross-linking and thus, a reform expansion structure that creates a crunchy texture in fabricated foods results”. The effect of extrusion variables on the properties of extruded cereals has been extensively studied by González *et al.*, 2000; Mitchell and Areas, 1992).

2.9.3 Effects of Extrusion Conditions on Raw Materials

Extrusion cooking provides thermo-mechanical and mechanical energy to cause physico-chemical transformations (starch gelatinization, melting and fragmentation reactions) of raw materials (Blanche and Sun, 2004; Anton and Luciano, 2007). The application of physical methods in characterizing the extrudate provides an in-depth knowledge on the extent of material transformation during extrusion cooking.

During extrusion cooking the food material undergoes physical and chemical changes such as gelatinization and break down of starch, denaturation of proteins and complex formation between lipids and amylose due to the high temperature and high shear stress

(Llo *et al.*, 2000; Caltinoglu *et al.*, 2013). The physical characteristics of the extrudate reflect the effectiveness of the process and suitability of ingredients (Patil *et al.*, 2005). The compositions of protein, lipid and starch as well as type of raw materials are important in controlling expansion, volume and extrudate quality (Chinnaswamy and Hanna, 1988a)

Native lipids might be present in the raw materials or added during extrusion (Singh *et al.*, 2007). Increased levels of lipids emerging from high-fat materials during extrusion impede the extruder performance (Camire, 2000a). The formation of free fatty acids can be prevented by extrusion cooking by the denaturation of hydrolytic enzymes (Camire *et al.*, 1990). Lipid oxidation which can occur after extrusion may result in off flavour and imparts negatively on the sensory attributes. Oilseeds may contain up to 50% by total seed weight as oil; hence the need to de-fat either partially or wholly before extrusion is advised (Singh *et al.*, 2007).

Effects of extrusion processing condition on the product qualities also have been extensively studied by Chiu *et al.*, 2012; Caltinoglu *et al.*, 2013; Sanchez-Madrigal *et al.*, 2014; Thachil *et al.*, 2014). Extrusion cooking can be affected by the physical size and shape of the starch granules. Starch is totally gelatinized during extrusion while destroying inherent antinutritional factors (Ravindran *et al.*, 2011). Smaller starch granules have equally shorter distance of heat transfer from in raising temperature to critical melting point. Hence the dough softens speedily in the extruder barrel. A second effect is observed in low moisture conditions (14-16%) owing to the physical starch granule shape.

Extrusion process improves soluble dietary fibre and reduces lipid oxidation. Transformations in proteins and amino acid profile, carbohydrates, vitamins, dietary fibre and mineral contents of foods occur during extrusion cooking (Singh *et al.*, 2007).

Areas, (1992), have investigated the effects of the raw materials and extrusion processing conditions on the nutritional quality of final food product.

Maillard also known as non-enzymatic reaction is a chemical reaction involving free amino groups and carbonyl groups commonly found in many foodstuffs resulting in the formation of brown pigments known as melanoids and production of flavours (Singh *et al.*, 2007). The occurrence of maillard reactions between proteins and sugars during germination process and extrusion cooking reduces the nutritional profile of the product. It is essential to note that the extent of reduction of nutrients during germination and extrusion cooking depends on the nature of raw material used. Nutritional changes such as changes in carbohydrates, protein and amino acid profile, mineral content and dietary fibre may be favourable or unfavourable as this result in the reduction of the availability of amino acids involved in protein digestibility (Singh *et al.*, 2007).

2.9.4 Chemical reactions resulting from Extrusion Cooking

Starches in general are known to contain some amounts of lipids and depending on the plant source; lipids in the starches vary widely (Ottenhof and Farahnaky, 2004). “Lipids are components that play an important role in most of the extrusion cooking processes” (Llo *et al.*, 2000). Lipid complexation with starch is a very important reaction in extrusion cooking that affects structure formation and texture of the extruded [products (De Pilli *et al.*, 2011). Lipids found in cereal starches are primarily in the form of free fatty acids and lysophospholipids (Buleon *et al.*, 1998). According to Ottenhof and Farahnaky, (2004), these lipids present in the starches of most plants, have the tendency of forming complexes with amylose to form amylose-lipid complexes. The complex consists of three amylose helixes with each comprising of six glucose units.

During extrusion cooking, the native structure of amylose is partially destroyed, and new crystalline ones corresponding to the amylose-lipid complex are formed (Merayo *et al.*,

2011). The formation of amylose-lipid complex during extrusion cooking has an important influence on structure, texture and other functional properties of the puffed extrudates (Bhatnagar and Hanna, 1997; Desrumaux *et al.*, 1999). Thachil *et al.*, (2014) reported that the incorporation of lipids in starch-based food products changes its physicochemical properties favourably or unfavourably. And these physicochemical changes brought about in starchy foods have been attributed to the formation of complexes between amylose and lipids (Singh *et al.*, 1998; De Pilli *et al.*, 2011).

Although it is uncertain that amylose-lipid complex is formed in native starch or cooked starch, Blanshard *et al.*, (1987) indicated that the amylose-lipid complexes are formed within the native starch granule. However, Le Bali *et al.*, (1999) proved that the amylose-lipid complex is formed upon heating of starch samples; and suggested that heating can cause some of the inherent amylose-lipid complex to aggregate together to form crystals. Caltinoglu *et al.*, (2013) suggested that the amylose-lipid complex results in difficulty in differentiating the influences of individual variables on the changes in final product characteristics.

The extrusion cooking process denatures undesirable enzymes; inactivates some antinutritional factors (trypsin inhibitors, haemagglutinins, tannins oxalates and phytates); sterilizes the product; and is responsible for the retention of natural colours and flavours in foods (Bhandari *et al.*, 2001). The enzyme hydrolysis of protein has been reported to improve after extrusion cooking due to the inactivation of antitrypsin activity in extruded snacks (Singh *et al.*, 2007).

The effects of various processing condition on lysine retention is very important since lysine is the most limiting essential amino acid in cereal-based products (Singh *et al.*, 2007). Llo *et al.*, (2000) reported that increased temperature during extrusion promotes

the degradation of inherent natural antioxidant while the formation of other antioxidants resulting from maillard reactions could be expected.

Chemical reactions such as gelatinization of starch, denaturation of proteins, maillard reactions occur during extrusion-cooking process which strongly influence the viscosity function (Moscicki and Van Zuilichem, 2011). Singh *et al.*, (2007) reported that maillard reaction occurs between free amino groups of protein and carbonyl groups of reducing sugars, resulting in decrease availability of amino acids and protein digestibility. Also, process conditions applied in extrusion cooking (high barrel temperatures and low feed moistures) encourages maillard reaction. Other non-nutrient healthful components of foods resulting from extrusion cooking are phenolic compounds (genistein and phytoestrogens), glucosinolates and Isoflavones,

2.9.5 Effects of Extrusion Cooking Conditions on Cowpea

Several works involving extrusion cooking of wheat, corn, rice and some legumes such as cowpea, peas, pea nut and soya bean has been done by many authors notably Kaur and Singh, 2000; Hagenimana, *et al.*, 2006; Mbaeyi and Onweluzo., 2010; Kumar *et al.*, 2010; Asare *et al.*, 2011; Filli *et al.*, 2012; Singh *et al.*, 2012 and Obadina, *et al.*, 2013.

Results from a study by Asare *et al.*, (2011) revealed that addition of legumes (Cowpea and peanuts) significantly increased the protein, fat, ash and all physico-functional properties; suggesting the use of legumes in improving the nutritional value of cereal blends. Significant increase in phosphorus, sodium and potassium content of sprouted samples and a decrease of calcium in the pre gelatinized samples were observed when sprouted sorghum grains were mixed with pigeon pea and extruded as breakfast cereal (Mbaeyi and Onweluzo, 2010). There was marginal increase in expansion ratio as the addition of cowpea flour heightened (Filli *et al.*, 2012).

The bulk density values measure in a study on the effect of extrusion conditions on the physicochemical properties and sensory characteristics of millet-cowpea based fura by Filli *et al.*, (2012), varied from 0.1 – 0.4 kgm³ for both samples (10% cowpea, 20% feed moisture and 250 rpm screw speed 20; 20% cowpea, 25% feed moisture and 284 rpm screw speed and 20% cowpea, 33.4% feed moisture and 200 rpm screw speed) respectively.

Ravidran *et al.*, (2011) conducted a study on evaluating the effects of three galactomannans on the physical, nutritional characteristics and sensory acceptability of pea-rice based extruded products. The rice-pea blend was in the ratio of 30:70 with guar gum (GG), locust bean gum (LBG) and ferrugreek gum (FG). The results showed that all three gums resulted in good expanded products; increasing the inclusion of gums however ($p > 0.05$) had no effect on the degree of expansion. Also, the addition of 5% guar gum and locust bean gum reduced ($p < 0.05$) the hardness of extruded products. Ferrugreek gum produced extrudates that were harder and crispier.

The high content of proteins in cowpea enzyme hydrolysis during extrusion cooking conclusively has been known to improve proteins nutritionally (Singh *et al.*, 2000). Singh *et al.*, (2007) reported that enzymes hydrolysis of protein is improved after extrusion cooking due to the inactivation of trypsin activity in the extrudates; extrusion cooking has been observed to improve pepsin hydrolysis due to denaturation of proteins rendering them more susceptible to pepsin activity. Additionally, studies have shown that rate of protein digestibility is higher in extruded products compared to non-extruded ones due to the denaturation and inactivation of antinutritional factors that may retard the digestion process (Singh *et al.*, 2007).

2.9.6 Effect of Extrusion Variables on Product Quality

It was reported in a study by Thachil *et al.*, (2014) that increased amylose content in feed mixture resulted in crispy extrudates with significantly higher ($p < 0.05$) amylose-lipid complex formation, greater radial expansion, lower hardness and higher oxidative stability. Amylose-lipid complex formation and its complimentary effect, thus, oxidative stability was significantly higher in extrudates incorporated with coconut oil compared to those with fish. Increased levels of fatty acids resulted decrease in WSI when carrot pomace and rice flour blend was extruded as reported Kaur and Singh (2000). Kumar *et al.*, (2010) and Suksomboon *et al.*, (2011) reported of similar decreased in WSI values when the feed moisture content of rice-soya blend were increased and extruded. In a related experiment by Siddiq *et al.*, (2013), similar results was obtained when a twin-screw extruder was used in extruding navy and pinto bean flours for gluten-free products. A significant increase in water absorption index (WAI) from 2.10 in steam-cooked (STC) to 2.73 in extruded (EXT) navy flour and water solubility index (WSI) of 13.33 to 25.56 (navy) and 13.35 to 21.19 pinto were measured.

Although, about twice the WAI indices measured in the extruded navy and pinto beans was measured in the cowpea-enriched extrudates; the WSI values of the cowpea-fortified extrudates slightly decreased from that of the extruded pinto and navy beans (Siddiq *et al.*, 2013). Extrusion cooking of high amylose rice has been reported to result in low WSI. Similar results were observed by (Pan *et al.*, 1992) when low WSI values were recorded among amylose-rich rice samples. The extrusion cooking was reported by Siddiq *et al.*, (2013) to have affected the redness (a^*) and yellowness (b^*) values significantly in navy and pinto bean flours whereas a mixed trend was observed for lightness (L^*) values.

Expansion is known to be a complex phenomenon that results from myraid of factors and mexhanisms influenced by composition of feed and processing conditions of extrusion (Patil *et al.*, 2007). Expansion leading to puffing of products is desirable in extruded products and is an important parameter regarding consumer acceptability (Anto and Luciano, 2007). Expansion encourages dehydration and the development of a crispy texture on the final extrudate which is mostly desirable (Patil *et al.*, 2007), and starch is the primary component of extruded products like expanded snacks and puff cereals and is responsible for majority of their mechanical properties (Ravindran *et al.*, 2011).

2.9.7 Conclusion on Literature Review

In spite of the numerous studies done on legume-cereal complementation in extrusion cooking, little has been done on the use of sprouted legumes (such as cowpea) and cereal in extrusion cooking of breakfast cereals. The study addressed the need to evaluate the outcome of extruded cereals from sprouted cowpea-rice flour. Specifically, physical and rheological characterizations of the extrudates as well as the molecular characterizations of the protein fractions were studied. Human and instrumentation sensory approach was applied in evaluating its consumer acceptance



CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Raw Materials

Two cultivars of rice (*Viwonor* and *Jasmine 85*) and one cultivar of cowpea (*Vigna unguiculata*) were obtained from Ghana Rice Inter Professional Body (GRIB), Ghana and the open market respectively.

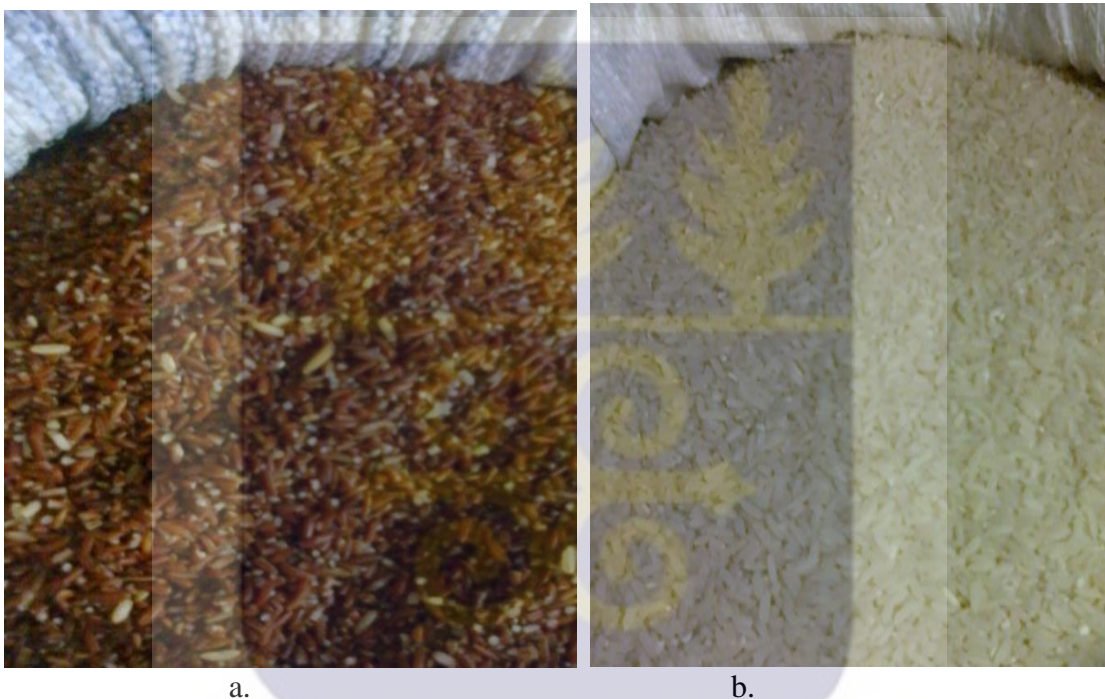


Plate 3.1: Images of rice samples a (*Viwonor*) and b (*Jasmine 85*)

3.2 Preparation of Raw Materials

The rice samples (*Viwonor* and *Jasmine 85*) were milled into flour using a disc attrition mill (Agrico Model 2A, New Delhi, India). The cowpeas were decorticated following the method described by Sefa-Dedeh *et al.*, (2001). Briefly, the cowpeas were cleaned, submerged in water for 3 minutes (to loosen the seed coat) at room temperature and dried in the convection oven (Astell Scientific) at 50⁰C for 18 hours. The dried cowpeas was passed through a disc attrition mill with its plates adjusted such that they were just wide enough for the cowpea grains to be split into cotyledons. Afterwards, the seed coats

were separated from the cotyledons using a seed cleaner (ALMACO Allan Machine Company, 99 M AVE NEVEDA IOWA 50201 U.S.A.). Another batch of whole cowpeas was soaked in water for 40 minutes and spread on pre-sterilized, water soaked jute sacks. They were left to sprout for 1, 2 and 3 days, with daily sprinkling of water on them to keep them moist. They were dried in the convection oven at 50⁰C for 18 hours and dehulled using the disc attrition mill, following the method described by (Sefaddeh *et al.*, 2001). The decorticated cowpeas were milled into flour using the disc attrition mill (Agrico Model 2A, New Delhi, India). Rice and cowpea flour samples were sealed in polyethylene bag and stored at room temperature prior to analysis.

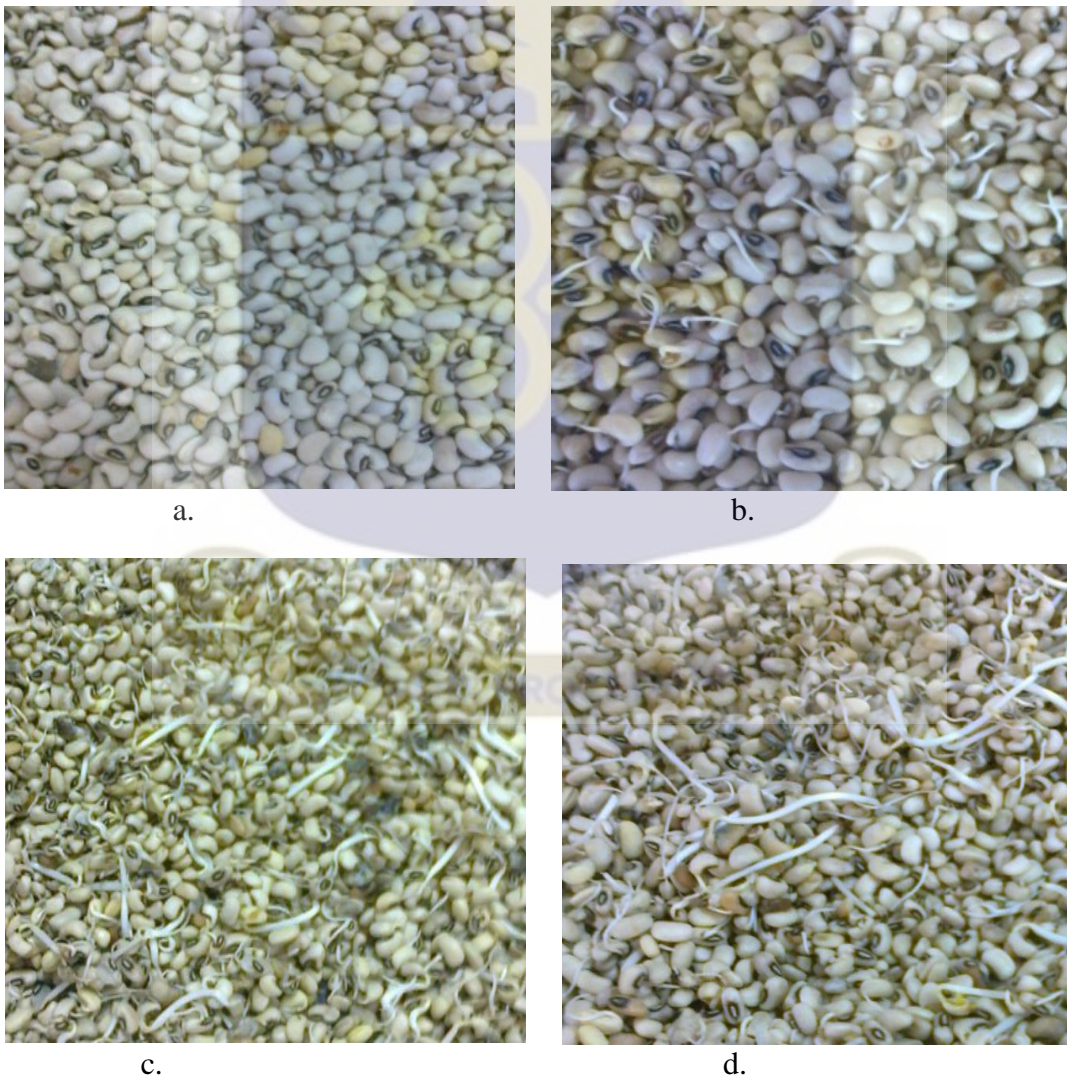


Plate 3.2: Images of sprouted cowpea; a (unsprouted cowpea/0 day sprouted cowpea), b (1 day sprouted cowpea), c (2 day sprouted cowpea), and d (3 day sprouted cowpea).

3.3 Physico-Chemical Analysis on Milled Rice

3.3.1 Milling Recovery (% Head Rice)

Milled rice samples (*Viwonor* and *Jasmine 85*) obtained was separated into whole and broken grains using a Test rice grader. The milling recoveries (% head rice) were then estimated using the following equations (Graham-Acquaah *et al.*, 2015).

$$\text{Head rice ratio (\%)} = 100 \times \frac{\text{weight of head rice}}{\text{weight of milled rice}}$$

3.3.2 Grain dimension and chalkiness

Grain dimensions and chalkiness were estimated using the S21 Rice Statistic Analyzer, (LKL Technologia, Brazil). The equipment was run with a classificatory S21 version y4.05 software. The S21 was calibrated using a reference sample supplied by the manufacturer. The fluorescent light on the S21 was turned on and approximately 50g of whole grains weighed and emptied into its sample receiver. The ‘long white’ classification set up was opened in the capture mode on the software. The equipment was then switched on to vibrate and cause the release of individual grains from the receiver to slide on a blue tile background and pass beneath the attached camera that captured images of the grains. When all the grains had exited the receiver the image capturing mode was stopped. The grain dimensions were determined by processing the captured images and applying the ‘advanced filter-length distribution’ on the software. The grain length and width were then recorded and the length/width ratio calculated. To determine chalkiness, ‘the basic filter – chalky distribution’ was applied. The % total chalky area for the samples were recorded and reported as the percentage chalkiness of the samples.

3.3.3 Grain Hardness

Grain hardness was measured using a grain hardness tester (Fujihara Seisakusho LDT, Japan) as described by (Fofana *et al.*, 2011). Ten grains were used for each sample. The handle of the equipment was initially turned anti-clockwise to make room to place a grain on the sample table. Subsequently, the handle was turned clockwise until a cracking sound was heard. At this time, the black pointer returned to the zero point and the reading of the red pointer (kg) indicated the hardness of the grain.

3.3.4 Alkali Spreading Value (ASV)

ASV was determined using the method developed by Little *et al.*, (1958) which involves visual observation of the degree of dispersion of grains of the milled rice after their immersion in 1.7% potassium hydroxide solution (KOH). Approximately 10 ml of 1.7% KOH solution was poured on 6 rice grains placed in a transparent petri dish and incubated at room temperature for 23 hours after which the samples were observed and visual scores assigned (Jennings *et al.*, 1979). Based on the alkali spreading value, the gelatinization temperature (GT) of samples could be indirectly determined.

3.4 Physico-Chemical Analysis of Rice Flour

3.4.1 Moisture Content

Two grams of finely ground samples was weighed into dried and weighed crucibles. Samples were dried in an oven (Termostabil C3, Ovenlab, Italy) for 90 mins at a temperature of 130°C. It was allowed to cool for an hour in the desiccator and weights were recorded. Measurements were performed in duplicate and differences in sample weight before and after drying were used to calculate the moisture content of the samples.

3.4.2 Apparent Amylose Content

Amylose content was measured using the standard iodine colorimetric method ISO 6647-2-2011. Ethanol (1 mL, 95%) and 1 M sodium hydroxide (9 mL) was added to flour (100 mg) and the mixture was heated in a boiling water bath until gelatinization of the starch occurred. After cooling, 1 M acetic acid (1 mL) and iodine solution (2 mL) were added and the volume made up to 100 mL with Millipore water. The iodine solution was prepared by dissolving 0.2 g iodine and 2.0 g potassium iodide in 100 mL Millipore water. Absorbance of the solution was measured using an auto Analyzer 3 (Seal Analytical, Germany) at 600 nm. Amylose content was quantified from a standard curve generated from absorbance values of 4 well-known standard rice varieties (IR65, IR24, IR64 and IR8).

3.4.3 Molecular Characterization of Protein Fraction in Rice Flours

The molecular characterizations of proteins in foods allow the estimation of the formation and stability of protein networks upon processing and cooking (Bonomi *et al.*, 2012). These approaches are also used in the evaluating the accessibility of specific residues that are relevant in the formation of protein network. Structural characterizations of proteins in various samples are performed by evaluating their solubility in various media. The buffers used show a different dissociating ability of covalent and non-covalent inter protein bonds that represent a useful index to describe the overall protein organization in extrudates obtained (Bonomi *et al.*, 2012). The nature of the proteins solubilized in the different media from the various samples are investigated by SDS-PAGE but the pattern of individual polypeptides are very low traces and similar according to the starting protein content (rice and cowpea flour).

3.4.3.1 Protein Solubility of Rice Flours

The solubility of proteins of rice and cowpea samples in native and denaturing conditions were determined by suspending 0.5g of finely ground samples in 5 ml of 0.05 molL⁻¹ mM sodium phosphate dihydrate (NaHPO₄), 0.1 molL⁻¹ mM sodium chloride (NaCl) of pH 7.0, NaHPO₄/NaCl containing 8 molL⁻¹ urea and 8 molL⁻¹ urea with 0.01 molL⁻¹ dithiothreitol (DTT), where indicated (Iametti *et al.*, 2006). After 1 h stirring at 25 °C, the suspensions were centrifuged (10,000 x g for 30 min at 20 °C). The amount of protein in the supernatant was determined by the dye-binding method (Bradford, 1976) using bovine serum albumin as a standard and Coomassie brilliant blue as the dye. Results were expressed as mg proteins/g sample.

3.4.3.2 Preparation of Bradford Reagent (Coomassie Brilliant Blue)

100 mg of Coomassie Brilliant Blue, CBB G-250 was dissolved in 50 ml 95% ethanol. 100 ml of concentrated sulphuric acid (H₂SO₄) and 850ml of distilled water was added to the resulting solution. The solution was stirred using a magnetic stirrer overnight and filtered into a tinted glass bottle that was wrapped in aluminum foil.

3.4.3.3 Construction of Standard Curve and Determination of Protein Quantity

The protein standard used for the Bradford assay was Bovine Serum Albumin (BSA). Protein solution containing 1 to 10 ug of protein in a volume up to 0.1 ml was pipetted into eppendorf tubes. The volume in the eppendorf tubes was adjusted to 0.1 ml with appropriate buffer used in solubilizing the protein samples in the above stated buffers. 1 mL of protein reagent (Bradford solution) was added to the test tube and the contents were thoroughly mixed on vortex. A blank solution was prepared with 0.1ml of the appropriate buffer and 1ml of the protein reagent (Bradford solution) added. Absorbance at 595 nm was read after 10 min using 3 ml plastic cuvettes against the blank. The

weight of protein (μg) was plotted against the corresponding absorbance resulting in a standard curve, and was used to determine the protein content in the samples.

3.4.3.4 Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) of Rice Flours

Solubilized proteins from rice and cowpea samples were diluted with denaturing buffer (0.125M Tris-HCl pH 6.8, 50% Glycerol (w/v), 1.7% SDS (w/v), 0.01% Bromophenol Blue (w/v) containing 2.5% 2-mercaptoethanol (v/v). The solution was heated at $100\text{ }^{\circ}\text{C}$ for 10 min and allowed to cool. Gel electrophoresis was performed according to the method described by Laemmli (1970) in denaturing conditions (0.1% SDS) and in the presence of a reducing compound (2-mercaptoethanol),

Electrophoretic separation was performed by using a 12% (w/v) acrylamide separating gel, where samples were loaded to a 4.5% (w/v) acrylamide stacking gel. Gels were prepared by mixing a 30% acrylamide solution (acrylamide/bis-acrylamide ratio 37.5:1), 1.5 M Tris-HCl buffer, pH 8.8, 0.4% SDS (separating gel), and 0.5M Tris-HCl buffer, pH 6.8, 0.4% SDS (stacking gel). Gel polymerization was catalyzed by adding 5% ammonium per sulfate, and N, N, N', N'-tetramethylethylenediamine (TEMED).

Electrophoresis run was performed by using a buffer system at pH 8.3 (0.025M Tris-HCl, 0.192M glycine, 0.1% (w/v) SDS) in a Miniprotein II (Mini Protein apparatus, BioRad, Richmond, VA, USA Bio Rad) apparatus, at a constant rate of 16 mA per gel. After the electrophoretic run, the gel was stained by immersion into a solution of the following composition: 30% (v/v) ethanol, 10% (v/v) acetic acid, 0.05% (w/v) Coomassie Brilliant Blue R250, 0.1% (w/v) copper (II) sulfate. After staining, the gels were destained by immersion into a solution containing 30% (v/v) ethanol, and acetic acid 10% (v/v). Images of the gels were scanned and observed using the EPSON 550

scanner. Protein molecular weight was determined by running on the same gel molecular weight markers (Pharmacia Low molecular weight) in the range 14-94 kDa.

3.4.4 In vitro Pepsin Digestion of Rice Flours

1.0 g of grounded samples was inserted into polypropylene test tubes and 10 ml of 0.05 M HCl added. Proteins were hydrolyzed by gastric pepsin (porcine stomach mucosa, EC 232-629-3, ref P7012, Sigma) at 1:2000, pepsin: protein ratio for 60 min at 37 °C under mixing conditions. In vitro pepsin digestion was terminated by the addition of 10% (final concentration) trichloroacetic acid after 60 min. Samples were then centrifuged at 13000g for 20 min, and the hydrolyzed peptide content in the supernatant was measured at 280 nm.

3.4.5 In vitro Pancreatin Digestion of Rice Flours

Protein hydrolysis from pancreatin was preceded by pepsin digestion for 60 min at 37°C as described previously. The pH of samples was adjusted to about 8.0 by adding TRIS 1 M. Proteins were hydrolyzed by pancreatic enzymes (pancreatin from porcine pancreas, EC 232-468-9, ref P1625, Sigma) at a 1:200 pancreatin: protein ratio for 180 min at 37 °C under mixing conditions. Pancreatin digestion was terminated by the addition of 10% (final concentration) trichloroacetic acid after 60, 120 and 180 min. Samples were then centrifuged at 13000g for 20 min, and the hydrolyzed peptide content in the supernatant was measured at 280 nm.

3.4.6 Accessible Thiols in Rice Flours

Accessible thiol groups were determined by suspending 0.25 g of finely ground samples in 5 mL of 50 mM sodium phosphate, 0.1 M NaCl, pH 7.0, in the presence/absence of 6 M urea, containing 0.2 mM 5,5-dithiobis-(2-nitrobenzoate) DTNB, (Ellman, 1959). After 1 h stirring at room temperature, the suspension was centrifuged (~13000g, 30 min, 25 °C) and the absorbance of the supernatant read at 412 nm. The blank was

prepared by suspending 0.25 g of samples in 5 mL of 50 mM sodium phosphate, 0.1 M NaCl, pH 7.0, in the presence/absence of 6 M urea, without adding DTNB. Results were expressed as μmol thiols/g rice.

3.5 Physico-Chemical Analysis of Cowpea Flours

3.5.1 Moisture Content of Cowpea Flours (as described in section 3.4.1).

3.5.2 Protein Solubility of Cowpea Flours (as described in section 3.4.3.1).

3.5.3 Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) of Cowpea Flours (as described in section 3.4.3.4).

3.5.4 In vitro Pepsin Digestion of Cowpea Flours (as described in section 3.4.4).

3.5.5 In vitro Pancreatin Digestion of Cowpea Flours (as described in section 3.4.5).

3.5.6 Accessible Thiols in Cowpea Flours (as described in section 3.4.6).

3.6 Analysis on Rice- Cowpea Flour Formulations

3.6.1 Pasting properties of Rice-Cowpea Flour Formulations

The pasting properties of rice and cowpea formulations were measured in a Brabender Micro Visco AmyloGraph, MVAG (Brabender OHG, Duisburg, Germany). 15 g of each formulation were dispersed in 100 ml of distilled water at 14% moisture content. The temperature profile consisted of a heating step from 30 up to 95 °C, at a rate of 3 °C/min, and then slurries were held for 20 min at 95 °C, allowed to cool to 30 °C, and maintained at this temperature for 1 min. The pasting properties of the raw formulations were evaluated in duplicate under constant conditions (speed: 250 rpm; sensitivity: 300 cm gf) according to Marti *et al.*, (2010).

3.7 Formulations of Flours and Extrusion Cooking

3.7.1 Design of Experiments

Full factorial experimental design was used in the rice-cowpea flour formulations. At the preliminary stage, two factors were considered; sprouting time (4 levels = 0, 1, 2 and 3 days) and cowpea: rice ratio (5 levels = 30, 40, 50, 60 and 70%). A total of 20 flour mixtures were obtained at the preliminary stage.

3.7.2 Formulation of Rice- Sprouted Cowpea Flour

A Central Composite Rotatable Design (CCRD) of $k=2$ for two factors (sprouting time and cowpea ratio) was varied in three coded levels. Each variable was evaluated at a high (+1), low (-1) and a central level (0). Table 3.1 describes the experimental design. The variables were set for 13 experiments consisting of 8 experimental runs with additional 5 runs at the centre point level to check for reproducibility. Central Composite Rotatable Design matrix of sprouting time and cowpea ratio for 13 experiments for the study of two experimental factors; observed and predicted values are shown in Table 3.1

Table 3.1: Central Composite Rotatable Design matrix for 13 experiments

Independent Variables with Coded levels				Responses	
Run	Pt Type	Time -1	Cowpea – 1	Sprouting Time (Days)	Cowpea ratio (%)
1	1	1.00	70.00	0	70.00
2	-1	4.62	50.00	3	50.00
3	0	2.50	50.00	2	50.00
4	-1	2.50	78.29	2	78.00
5	1	4.00	70.00	3	70.00
6	0	2.50	50.00	2	50.00
7	1	1.00	30.00	0	30.00
8	0	2.50	50.00	2	50.00
9	1	4.00	30.00	3	30.00
10	-1	0.38	50.00	0	50.00
11	0	2.50	50.00	2	50.00
12	0	2.50	50.00	2	50.00
13	-1	2.50	21.70	2	22.00

Table 3.2: Compositions of 13 Flour Formulations

Sample	Code	Composition
1	Formulation 1	30% unsprouted cowpea + 70% <i>Viwonor</i> rice
2	Formulation 2	30% 3 day sprouted cowpea + 70% <i>Viwonor</i> rice
3	Formulation 3	70% unsprouted cowpea + 30% <i>Viwonor</i> rice
4	Formulation 4	70% 3 day sprouted cowpea + 30% <i>Viwonor</i> rice
5	Formulation 5	50% unsprouted cowpea + 50% <i>Viwonor</i> rice
6	Formulation 6	50% 3 day sprouted cowpea + 50% <i>Viwonor</i> rice
7	Formulation 7	22% 2 day sprouted cowpea + 78% <i>Viwonor</i> rice
8	Formulation 8	78% 2 day sprouted cowpea + 22% <i>Viwonor</i> rice
9	Formulation 9	30% 2 day sprouted cowpea + 70% <i>Viwonor</i> rice
10	Formulation 10	50% 2 day sprouted cowpea + 50% <i>Viwonor</i> rice
11	Formulation 11	50% 2 day sprouted cowpea + 50% <i>Viwonor</i> rice
12	Formulation 12	50% 2 day sprouted cowpea + 50% <i>Viwonor</i> rice
13	Formulation 13	50% 2 day sprouted cowpea + 50% <i>Viwonor</i> rice

3.7.3 Extrusion Cooking

A twin screw (co-rotating) extruder (CLEXTRAL BC 21) was used to extrude all 13 formulations (5 were centre points) under the following conditions: extruder barrel temperature at 200⁰C, extruder screw speed, 1200 rpm; diameter of die, 4.0 mm and at 25% moisture content. The extrudates were collected at for analysis when the extruder was at equilibrium. The extrudates were allowed to cool at room temperature for 30 mins and were sealed in transparent polythene bags (Poly Products (GH) Ltd) prior to the analysis. The formulated flour samples were equally sealed in transparent polythene bags (Poly Products (GH) Ltd) for further analysis.

3.7.4 Consumer Preference Test of Extrudates

A consumer preference test was performed on the products by ranking extruded samples as described by Meilgaard, *et al.*, (1991). 50 students were recruited from the

department of Food Science and Technology of Kwame Nkrumah University of Science and Technology (KNUST) as consumers. The consumers ranked the extrudates on 9-point Hedonic scale (9 representing the highest degree of likeness and 1 representing the highest degree of dislike). 50 g each of the 13 extrudates were presented to the panelists in 100 ml clean disposable cups with 3-digit codes. The consumers were kept in separate booths (specially designed for sensory evaluation of food products) in the presence of optimum lightening. 500 ml of drinking water was provided to each panel and 13 sachets of 30 g powdered milk and granulated sugar were provided to each panelist. Sensory Evaluation questionnaires (Appendix 23) were provided additionally. Extrudates were assessed in terms of Colour, Aroma, Crunchiness, Taste and Overall Acceptability. The focus was on the overall acceptability scores. Appropriate scores for each attributes were provided on the ballot sheet as assessed by the panelists. The Analysis of Variance (ANOVA) was performed on the results of the consumer preference test and the mean acceptability scores was used to rank the extrudates. A common flour formulation ratio of 30% cowpea + 70% rice derived from the best 3 extrudates was used in extruding additional 3 extrudates from the high quality - *Jasmine* 85 rice as a control. In all 6 best extrudates were obtained.

3.8 Analysis on Rice-Cowpea Extrudates

3.8.1 Moisture Content of Extrudates (as described in section 3.4.1).

3.8.2 Protein Solubility of Extrudates (as described in section 3.4.3.1).

3.8.3 Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) of Extrudates (as described in section 3.4.3.4).

3.8.4 In vitro Pepsin Digestion of Extrudates (as described in section 3.4.4).

3.8.5 In vitro Pancreatin Digestion of Extrudates (as described in section 3.4.5).

3.8.6 Accessible Thiols in Extrudates (as described in section 3.4.6).

3.9 Analysis on Extrudate Quality Characteristics

3.9.1 Water Absorption Index (WAI) and Water Solubility Index (WSI)

WAI and WSI indices were measured using a technique developed for cereals (Anderson *et al.*, 1969). 2.5 g of ground sample were suspended in 25 ml of water at room temperature and, after 30 minutes of gently stirring, they were centrifuged at 3000 *g for 10 minutes. The supernatant liquid was poured carefully into a tarred evaporating dish. The remaining gel was weighed and the WAI calculated from its weight. The WAI is the weight of gel obtained per gram of dry sample. As an index of water solubility, the amount of dry solids recovered by evaporating the supernatant from the water absorption test was expressed as percentage of dry solids in the sample.

3.9.2 Bulk Density

The bulk densities of the extrudates were measured using the method described by Abd-Hady *et al.*, (2002). A 1 L cylinder was filled with sesame seeds, and the surface leveled with a ruler. A piece of extrudate – the volume of which was to be determined - was weighed and placed in a 1 L cylinder. The sesame seeds from the beaker were poured over the extrudates and leveled. The volume of the spilled sesame seeds was noted as the volume of extrudates. Bulk density of extrudates was expressed as the extrudate weight per unit volume of extrudate, and indicated as g/cm³.

3.9.3 Expansion Ratios

The cross-sectional diameter of the extrudates was measured with a Vernier caliper. The Expansion Ratio (ER) was calculated as the cross-sectional diameter of the extrudate divided by the diameter of the die opening (Ding *et al.*, 2005). The ER values were obtained from 15 random samples with three locations in each for each extrusion condition.

$$\text{Expansion Ratio} = \frac{\text{Diameter of Extrudate}}{\text{Diameter of Die}}$$

3.9.4 Colour

Colour parameters were determined on the raw materials and extruded samples after grinding them into flours. In particular, the following indices were measured by using a colorimeter Minolta CR-300 (Japan): L* (lightness index), a*(red/green; redness index), b* (yellow/blue; yellowness index). A glass petri dish was filled with sample and underwent measurements by using the “L, a, b” system. The colorimeter was calibrated against a standard white calibration plate (Y=92.8, x= 0.3137 and y= 0.3199). For each sample 5 measurements were performed and results are expressed as their average.

3.9.5 Texture Profile Analysis

Extrudates were punctured by using a Zwick Z005 testing machine equipped with a 100 kN load cell (Zwick GmbH & Co., Ulm, Germany), fitted with a 4-mm diameter cylindrical flat-faced probe. The speed of advance was 1 mm s⁻¹. The force-deformation curves were recorded from the surface of the extrudate and ended after the pin had reached the 50% of sample deformation. The work (N*mm) divided by the height of the sample (mm) was chosen to represent the textural properties of extrudates that had different diameters. 20 measurements were carried out for each sample.

3.9.6 Porosity of Extrudates (Image Analysis)

Image acquisition

For each extruded sample, a total of 12 images were acquired and analyzed. During the acquisition process performed with a flatbed scanner (Epson Perfection 3170 Photo, Seiko Epson Corp., Japan), samples were covered with a black box to prevent loss of

light and images were acquired at a resolution of 600 dpi (dots per inch) and a colour depth of 24 bits.

Processing of Images

The captured images were saved in uncompressed TIFF format. To create the final data set of images, a region of interest (ROI) of 2068 * 3220 pixels was extracted from each single image. The ROI images were converted to 8 bit grey scale and subjected to spatial calibration before the analysis. For the morphological characterization of the bubbles, area (mm^2), diameter (mm), circumference (mm) and porosity (%) were measured. The bubbles, moreover, were classified into three different size classes according to their surface:

Class 1: bubbles area between 0.01 and 0.1 mm^2 ;

Class 2: bubbles area between 0.1 and 3 mm^2 ;

Class 3: bubbles area greater than 3 mm^2 .

3.10 Instrumental Sensory Evaluation

3.10.1 Electronic Nose

The electronic nose is the common name of electrochemical sensor systems responding to flavour/odour (volatiles) using an array of simple and non-specific sensors and a pattern recognition software system. Essentially, each odour leaves a characteristic pattern or fingerprint on the sensor array and pattern recognition able to distinguish and recognize the odours.

A Portable Electronic Nose (PEN2) from Win Muster Airsense (WMA) Analytics Inc. (Schwerin, Germany) was used. It consisted of a sampling apparatus, an array of chemical gas sensors producing an array of signals when confronted with a gas, vapour, or odour, and appropriate pattern-recognition software (Win Muster v.1.6) for data

recording and elaboration as shown in Figure 3.1



Fig 3.1: Electronic Nose PEN2

The key principles involved in the electronic nose concept, is the transfer of the total headspace of a sample to a sensor array that detects the presence of volatile compounds in the headspace and a pattern of signals is provided that are dependent on the sensors selectivity and sensitivity and the characteristics of the volatile compounds in the headspace (Fig. 3.2).

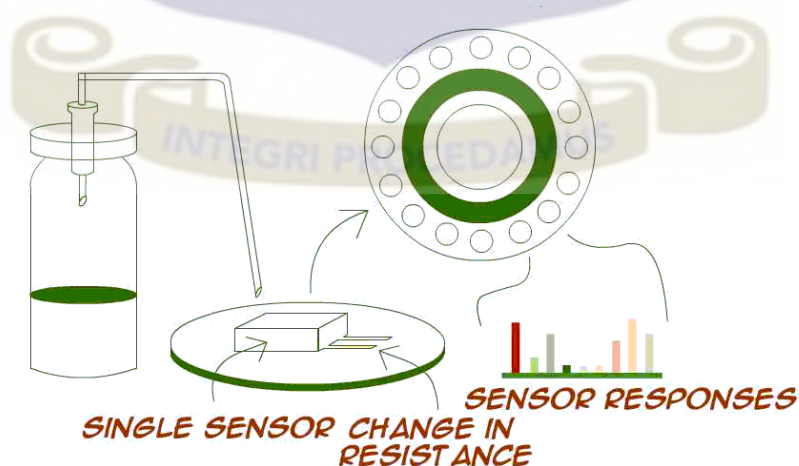


Fig 3.2: Basic Element of an Electronic Apparatus

By chemical interaction between odour compounds and the gas sensors, the state of the sensors is altered giving rise to electrical signals which are registered by the instrument. In this way the signals from the individual sensors represent a pattern which is unique for the gas mixture measured and is interpreted by multivariate pattern recognition techniques. Samples with similar odorous compounds generally give rise to similar sensor response patterns and samples with different odours show differences in their patterns.

The sensor array of the electronic nose PEN2 is composed of 10 Metal Oxide Semiconductor (MOS) type chemical sensors: W1C (aromatic) W5S (broadrange) W3C (aromatic) W6S (hydrogen) W5C (arom-aliph) W1S (broad-methane) W1W (sulphur-organic) W2S (broad-alcohol) W2W (sulph-chlor) W3S (methane-aliph). The sensor response is expressed as resistivity (Ω).

3.10.2 Operation Procedure on Electronic Nose

For the measurements, 0.2g of lyophilized samples was placed in a 40 mL airtight glass vial fitted with a pierceable Silicon/Teflon disk in the cap. After an hour, headspace equilibration at room temperature, the measurement sequence started. Operating conditions were: flow rate (300mL/min); injection time (60 min); and flush time (180 min) during which the surface of the sensors was cleaned with air filtered through active carbon. All samples were analyzed twice and the average of the sensor responses was used for subsequent statistical analysis.

Statistical Analysis

The multivariate output data generated by the gas sensor array signals represents another essential part of the electronic nose concept. The statistical techniques used are based on commercial or specially designed software using pattern recognition routines like Principal Component Analysis (PCA). Principal Components Analysis (PCA) is a

procedure that permits to extract useful information from the data, to explore the data structure, the relationship between objects, the relationship between objects and variables and the global correlation of the variables. It was used for explorative data analysis as it identifies orthogonal directions of maximum variance in the original data, in decreasing order, and projects the data into a lower-dimensionality space formed of a subset of the highest-variance components. The orthogonal directions are linear combinations (principal components PCs) of the original variables and each component explains in turns a part of the total variance of the data; in particular, the first significant component explains the largest percentage of the total variance, the second one, the second largest percentage, and so forth.

3.10.3 Electronic Tongue

Analyses were performed with the Taste-Sensing System SA 402B (Intelligent Sensor Technology Co. Ltd, Japan) namely Electronic Tongue (ET). The ET is a liquid analytical device that mimics the taste-sensing mechanism of gustatory system; it comprises two sensor arrays that are specific for liquid and are able to evaluate tastes: sourness, saltiness, bitterness, umami and astringency. The detecting part of the system consists of 7 sensors whose surface is attached with artificial lipid membranes having different response properties to chemical substances on the basis of their taste as reported in table 3.2.

Statistical Analysis

The taste values collected by electronic tongue (ET) were elaborated by Principal Component Analysis (PCA). PCA was used for explorative data analysis in order to achieve a partial visualization of the data set in a reduced dimension. PCA was performed in correlation (the variables were scaled). Two figures were obtained from the

elaboration; PCA-Score plot representing the relationship among the samples, and PCA-loading plot showing the relationship among the variables and how they influence the system.

The multivariate output data generated by the gas sensor array signals represents another essential part of the electronic nose concept. The statistical techniques used are based on commercial or specially designed software using pattern recognition routines like Principal Component Analysis (PCA). Principal Components Analysis (PCA) is a procedure that permits to extract useful information from the data, to explore the data structure, the relationship between objects, the relationship between objects and variables and the global correlation of the variables. It was used for explorative data analysis as it identifies orthogonal directions of maximum variance in the original data, in decreasing order, and projects the data into a lower-dimensionality space formed of a subset of the highest-variance components. The orthogonal directions are linear combinations (principal components PCs) of the original variables and each component explains in turns a part of the total variance of the data; in particular, the first significant component explains the largest percentage of the total variance, the second one, the second largest percentage, and so forth.

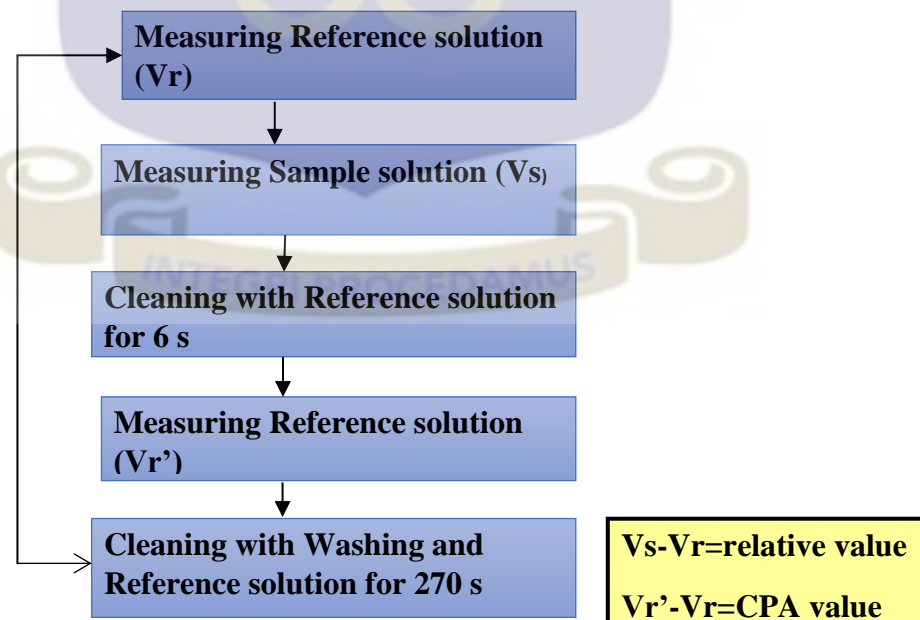


Fig 3.3: Electronic Tongue Apparatus

Table 3.3: Characteristics of Electronic Tongue Detecting Sensors.

Attribute	Name of detecting electrodes	Characteristics (Taste Information)
Blend Membrane	AAE	Umami Taste and Umami richness
	CTO	Saltiness
	CAO	Sourness
Positively charged membrane	COO	Bitterness and acidic Bitterness
	AE1	Astringency
Negatively charged membrane	AC0	Bitterness
	AN0	Bitterness

For this work a total of 5 detecting sensors and 2 reference electrodes were used, separated in two arrays according to membrane charge: hybrid (CTO; CA0;AAE) and positive (C00, AE1). The measurement principle of the electronic tongue (Fig. 3.4) is based on the capability of taste substances to change the potential of detecting sensors through electrostatic or hydrophobic interaction with the hydrophilic and hydrophobic groups of the lipid membranes. The response of each sensor, recorded as the difference between the potential detected by the sensor and the potential of the reference electrode, is elaborated by a computer and processed via a pattern recognition system.

**Fig 3.4: Electronic Tongue measuring process**

The detecting sensors and reference electrodes were first dipped into the reference solution (30 mM potassium chloride and 0.3mM tartaric acid) and the electric potential measured for each sensor was defined as V_r . Then the sensors were dipped for 30 s into the sample solution. For each sensor the measured potential was defined as V_s . For each sensor the “relative value” (R_v) was represented by the difference ($V_s - V_r$) between the potential of the sample and the reference solution. Sensors were rinsed with fresh reference solution for 6 s and then dipped into the reference solution again. The new potential of the reference solution was defined as V_r' . For each sensor, the difference ($V_r' - V_r$) between the potential of the reference solution before and after sample measurement is the CPA value (Change of Membrane Potential caused by Absorption) (CPA_v) and corresponds to the ET “aftertastes”. Before a new measurement cycle started, electrodes were rinsed for 90 s with a washing solution and then for 180 s with the reference solution. Each sample was evaluated two times and the averages of the sensor outputs were converted to taste information. The “taste values” were calculated by multiplying sensor outputs for appropriate coefficients based on Weber–Fechner law, which gives the intensity of sensation considering the sensor properties for tastes. In particular, the “taste values” were estimated as:

$$\text{Sourness} = 0.3316 \times R_v \text{ (CA0)}$$

$$\text{Saltiness} = -0.252 \times R_v \text{ (CT0)}$$

$$\text{Bitterness} = -0.140 \times R_v \text{ (C00)} + 0.084 \times R_v \text{ (CT0)}$$

$$\text{Aftertaste-bitterness} = -0.210 \times CPA_v \text{ (C00)}$$

$$\text{Astringency} = 0.1575 \times R_v \text{ (AE1)} + 0.1575 \times R_v \text{ (CT0)}$$

$$\text{Aftertaste-astringency} = -0.252 \times CPA_v \text{ (AE1)}$$

ANOVA was performed for all statistical analysis using MINITAB Version 14.0 where different superscript letters indicate significant difference.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Physical Properties of Rice

4.1.1 Head Rice

Head rice (whole grain) yield is the amount of whole grain recovered after milling a unit amount of paddy (Manful, 2013). Aside the cooking quality of head rice, it is a significant factor in determining yield. Significantly lower head rice ($p < 0.05$) was recorded in *Viwonor rice* (indicating higher percentage broken grains) which is a low quality index (Table 4.1). This may be due to the differences in the genetic composition, environment (Unnevehr *et al.*, 1992; Velupillai and Pandey, 1990; Fan, 1999; Singh *et al.*, 2003). Essentially, *Viwonor* and *Jasmine 85* belong to the *Glaberrima* and *Sativa* species of rice respectively, and are therefore very different. In some cases, conditions of milling, post milling handling and storage can be factors influencing the differences in percent head rice.

Manful, (2013) reported that milled rice can be classified into four groups depending on the total head rice obtained after milling. The classification includes Premium (above 57%), Grade 1 (48 to 56.9%), Grade 2 (39 to 47.9%) and Grade 3 (30 to 38.9%). Therefore based on the above classification, *Viwonor* rice can be classified under grade 1. *Jasmine 85* had 62.18 % head rice and can be classified as premium grade rice. This confirms the assertion that *Viwonor* rice is low grade rice with *Jasmine 85* being a superior cultivar.

4.1.2 Grain Chalkiness

Chalkiness in grain is described as the presence of whiteness on the edges or central part of the milled rice (Manful, 2013). It occurs at the early stage of grain development under

high temperature (Tashiro and Wardlaw (1991). The presence of chalky kernel adversely affects consumer acceptability. According to Codex Commission (1990), the tolerance limit for chalky grains is 11%; this clearly shows that at 0.9 %, one can confidently conclude that the presence of chalky grains in *Jasmine 85* is considerably low and acceptable. Table 4.1 indicates that *Jasmine 85* measured 0.9% chalky grains which make it good quality rice. The high consumer acceptability of the *Jasmine 85* can be linked to the report by Singh *et al.*, (2003) that rice containing above 2% chalky kernels is usually rejected in many global rice markets. Chalkiness in *Viwonor* could not be measured due to its dark-red colouration.

Further to the Codex classification for chalkiness in rice, Manful, (2013) set 5 classes of rice depending on the percentage of chalky grains measured in a sample of rice. These classes include Premium (below 2.1%), Grade 1 (2.1 to 5.0%), Grade 2 (5.1 to 10%), Grade 3 (10.1 to 15.0 %) and Poor (above 15 %). Based on the classification by Manful, (2013), *Jasmine 85* rice may be considered as 'high grade' rice which falls within the premium class due to the very low percentage chalkiness (0.9%) measured. The International Rice Research Institute IRRI, (2002) also documented 4 classes of chalky grains which include: none (0), small (below 10%), medium (11 to 20%) and large (above 20%); hence the percentage of chalky grains in *Jasmine 85* can be considered as small amount at 0.9%. The presence of chalky kernels in rice generally affects consumer acceptability and the low content of chalky grains in the *Jasmine 85* rice confirms its high consumer acceptability and patronage. It must be emphasized the percentage of chalky grains in the *Viwonor* rice could not be measured due to its appearance (dark red colouration).

Table 4.1: Physical Qualities of Rice

Rice Variety	Head rice fraction (%)	Chalky Grains (%)	Mean Grain Length mm	Mean Grain Width mm	Length/Width	Grain Hardness
<i>Viwonor</i>	48.07 ±2.51 ^a	-	6.54 ±0.05	2.13 ±0.14	3.1 ^a	8.65 ±0.28 ^b
<i>Jasmine 85</i>	62.18 ±0.13 ^b	0.9 ±0.20	6.56 ±0.04	2.25 ± 0.01	2.9 ^a	6.40 ±0.08 ^a

4.1.3 Grain Hardness

Rice hardness is one of the most significant factors influencing its eating quality (Webb *et al.*, 1986; Jiang *et al.*, 2010). Grain hardness is usually caused by internal cracking and is known to be important factor affecting rice grain quality (Velupillai and Pandey, 1990). Varietal differences significantly affected ($p < 0.05$) the hardness of the rice grain. Table 4.1 shows that *Viwonor* rice had a mean hardness index of 8.65 which was significantly higher than that of *Jasmine 85* at 6.40 hardness index. The differences in hardness index may be due to the differences in genetic composition, planting conditions and environmental influence (Singh *et al.*, 2003).

Several factors such as storage changes and aging, drying and handling, kernel appearance and translucency, resistance to insects, processing and grain breakage during milling have been attributed to grain hardness (Webb *et al.*, 1986). The relatively high hardness index in the *Viwonor* rice grains maybe due to the presence of fewer cracks. *Viwonor* rice has been known among many users to possess relatively longer cooking time. This may be due to its relatively higher hardness index.

4.1.4 Grain length (Size and shape)

The physical quality of rice is largely determined by the level of whole unbroken grains obtained. Grain length and shape are the physical quantities used in the classification of rice (Diako *et al.*, 2011). Grain length is defined as a measure of the longest dimension of the grain. The IRRI, (2002) categorized rice grain length or size into 4 classes which

include: Extra-long (above 7.5 mm), Long (6.6 to 7.5 mm), Medium (5.5 to 6.6 mm) and Short (5.5 mm or less). Judging from the above classification, one can deduce that *Viwonor* rice belonged to medium size class of rice (with grain length of 6.5 mm) while *Jasmine 85* can be classified under both medium and long grain class (with grain length of 6.6 mm). Essentially, Khush *et al.*, (1979) also developed rice grain size classification with the same dimensions of > 7.5 mm, 6.61-7.50 mm, 5.51-6.60 mm and < 5.50 mm for extra-long, long, medium and short respectively.

In reference to Table 4.2 *Viwonor* rice can be said to be slender in shape with *Jasmine 85* being medium. The grain shape (length/ width ratio) measured were 3.1 and 2.9 for *Viwonor* and *Jasmine 85* respectively. There were no significant differences in grain size ($p > 0.05$) between the two rice varieties. Manful *et al.*, (1998) noted that consumer acceptance and preference with respect to eating, cooking and processing qualities are important in judging rice quality since they vary from country to country. It is therefore possible for *Viwonor* rice to gain comparatively higher market value than *Jasmine 85* in areas where slender rice is preferred.

Table 4.2: International Standards for the classification of rice size and shape

Size		Shape	
Classification	Range	Classification	Range
Extra Long	Above 7.5 mm	Slender	Above 3.0
Long	6.6 to 7.5 mm	Medium	2.1 to 3.0
Medium	5.5 to 6.6 mm	Bold	1.1 to 2.0
Short	5.5 mm or less	Round	Less than 1.1

Source: International Rice Research Institute IRRI, (2002)

4.1.5 Colour of Rice Grain

Colour is known as a perceptual phenomenon that depends on the observer and the conditions in which the colour is observed (Pathare, *et al.*, 2013). Colour is defined by three characteristics namely hue, saturation and brightness. Hue is an attribute associated with the dominant wavelength in a mixture of light waves thus it represents the dominant colour as perceived by an observer. Saturation relative purity or the amount of white light mixed with a hue. Total colour difference (ΔE^*) is defined as the difference between two colors in an $L^* a^* b^*$ color space.

Varietal differences strongly affected the lightness of the rice. The dark red colour of the *Viwonor* rice reflected in its relatively lower lightness index (L^*) index and consequently higher redness (a^*) and yellowness (b^*) indices (Table 4.3). There was no difference in the total colour difference (ΔE^*) of the rice. Hue angle (h^*) is primarily the main parameter that defines appearance of a product and thus it represents the dominant colour perceived by an observer. The hue angle (h^*) was significantly affected ($p < 0.05$) by the cultivar of rice. This suggests that the overall appearance of the *Viwonor* rice was lower than that of the *Jasmine 85*.

Table 4.3: Colour Profile of Rice Flours

Sample	Lightness Index (L^*)	Redness Index (a^*)	Yellowness Index (b^*)	Total Colour Difference (ΔE^*)	Hue angle h^*
<i>Viwonor</i>	78.73 \pm 0.08 ^a	4.77 \pm 0.02 ^b	12.09 \pm 0.32 ^b	0.56 \pm 0.04 ^a	68.46 \pm 0.07 ^a
<i>Jasmine 85</i>	89.43 \pm 0.30 ^b	-0.27 \pm 0.06 ^a	9.08 \pm 0.28 ^a	0.58 \pm 0.19 ^a	88.32 \pm 0.67 ^b

4.2 Chemical Properties of Rice

4.2.1 Moisture Content (%) of Rice Flours

The determination of moisture content in food products is essential in the monitoring of the safety (microbial) and quality of the food. Moisture content was significantly influenced by rice cultivar ($p < 0.05$) with *Jasmine 85* measuring relatively lower moisture content (Table 4.4).

Table 4.4: Moisture Content (%) of Rice Flours

Sample	Moisture Content (%)
<i>Viwonor rice</i>	11.34 ± 0.47^b
<i>Jasmine 85 rice</i>	10.37 ± 0.12^a

4.2.2 Apparent Amylose Content

Amylose content is an important factor in determining the cooking and pasting behaviour of rice and its end use (Adu- Kwarteng *et al.*, 2003) and a rice quality determinant factor (Gibson *et al.*, 1997; Manful, 2013). Cooking and eating qualities of rice is mainly influenced by its inherent starch properties (Bao *et al.*, 2004). Amylose content in rice has been considered as important determinant of the quality of rice. Amylose content was significantly affected by rice variety ($p < 0.05$). Table 4.5 shows that *Viwonor* rice had intermediate amylose content (of 21.22 % on dry matter basis) while *Jasmine 85* recorded low amylose (of 16.89% on dry matter basis) content. According to Singh *et al.*, (2005), rice cultivars with high amylose tend to have less cooking time and are hard in texture; and this assertion confirms the high amylose content in the *Viwonor* rice with a resultant higher hardness index.

Table 4.5: Apparent Amylose Content of Rice Flours

Rice Variety	Apparent Amylose (%)	Apparent Amylose (%) on dry basis	Type of Amylose
<i>Viwonor</i>	18.82	21.22 ±0.06 ^b	Intermediate
<i>Jasmine 85</i>	15.14	16.89 ±0.04 ^a	Low

Juliano, (1992) classified amylose contents of 0-2, 5-12, 12-20, 20-25 and 25-33% as waxy, very low, low, intermediate and high amylose in rice respectively. Similarly, Manful, (2013), classified 0.2%, 3-9%, 10-19%, 20-24% and above 24% amylose as waxy, very low, low, intermediate and high amylose rice. The amylose content in rice has been classified by IRRI, (2002) as high (25-30%), intermediate (20-25%) and low (10-20%). Therefore, it can be concluded that *Viwonor* and *Jasmine 85* rice have intermediate and low amylose contents. Amylose rich rice has been found to absorb more water during cooking (FAO, 1993); hence *Viwonor* rice is most likely to absorb more water during cooking compared to *Jasmine 85*.

Amylose content helps the experimenter in classifying the samples into waxy and non-waxy. Waxy rice samples are known to be low in amylose whereas non-waxy rice is amylose rich. Waxy rice is usually flaky upon cooking while the non-waxy ones become sticky upon cooking. From the results, *Viwonor* rice can be said to be non-waxy (due to its relatively higher amylose content) and sticks together upon cooking.

Table 4.5 shows that amylose contents were significantly influenced by varietal differences ($p < 0.05$) in the two rice cultivars. The occurrence of variations in amylose content among different rice cultivars may be related to genetic differences as Ayres *et al.*, (1997) attributed the variation in amylose content in rice varieties to single

nucleotide polymorphism in an allele of the waxy gene encoding the granule-bound starch synthase (GBSS) enzyme. It can be inferred that the variations in amylose content in the two rice cultivars are likely to result from the differences in genetic composition. Behall and Scholfield (2005) reported that amylose plays an important role in reducing the glycemic and insulin impact of foods. It can therefore be inferred that *Viwonor* rice (with higher amylose content) has the tendency of possessing lower glycemic and insulin impact. Results on apparent amylose encourages the incorporation of *viwonor* rice in the production of cowpea-enriched breakfast cereal as amylose has been reported to be slowly digested and provides beneficial effects on human health (Hu *et al.*, 2010).

4.2.3 Alkaline Spreading Value (ASV)

The Alkaline Spreading Value (ASV) provides the Gelatinization Temperature (GT) class of the starches under study. Gelatinization temperature is the temperature at which rice starch gelatinizes or begins to melt upon heating; and it gives an indication of the cooking time of the rice. The measurement of alkaline spreading value gives an indication of the resistance of rice grains to alkaline digestion. Amylose content and gelatinization temperature are the most important quality indicators affecting textural characteristics (Koutroubas *et al.*, 2004).

Table 4.6: Alkaline Spreading Value of Rice Varieties

Rice Variety	ASV (Score)	Gelatinization Temperature Class
<i>Viwonor</i>	5.34	Intermediate
<i>Jasmine 85</i>	6.92	Low

Table 4.6 indicates that GT value for starch from *Viwonor* rice was higher (intermediate GT class) than that of *Jasmine 85*. This indicates that *Viwonor* rice has relatively higher

cooking time compared to *Jasmine 85*. Four gelatinization classes have been reported by Manful, (2013) as low, intermediate, high-intermediate and high. Therefore based on the above classification, starches from *Viwonor* and *Jasmine 85* rice cultivars exhibited intermediate and low Gelatinization Temperature Class respectively. Significant differences in gelatinization temperature were observed between the two rice varieties (Table 4.6).

Although studies by Juliano and Perez, (1990) reported that gelatinization temperatures are independent of amylose content for waxy and non-waxy rice starches, the relative higher cooking temperature recorded in *Viwonor* can be correlated to the higher hardness index recorded (Table 4.1). Tester and Morrison (1990) reported the relationship between starch granules and gelatinization temperature (GT) and reported that starches with low GT possess lesser crystallites than high GT starches. In view of this assertion, one can infer that *Jasmine 85* has a higher tendency of possessing less crystalline starch due to its low GT.

4.3 Characterizations of Protein Fractions in Rice Flours

4.3.1 Protein Content in Rice Flours

Protein contents in rice flours were assayed with saline, urea and Dithiothreitol (DTT) buffer. Rice variety significantly affected ($p < 0.05$) the quantity of proteins extracted in all buffers (Table 4.7). Relatively lower protein contents were measured in the *Viwonor* rice samples compared the *Jasmine 85* rice. The saline (neutral) buffer extracted the salt soluble proteins present in the rice samples while the urea buffer which is strongly basic solubilized the basic proteins. Extraction of proteins with the organic solute urea denatured more of the proteins in the samples that resulted in the extraction of more proteins. A further increase in protein content was measured with the DTT buffer in both rice samples (Table 4.7).

Table 4.7: Protein Content of Rice Flours

Sample	Buffer	Protein Content mg/g sample
<i>Viwonor</i>	NaCl	2.55 ±0.03 ^a
<i>Viwonor</i>	Urea	10.97 ±0.36 ^b
<i>Viwonor</i>	DTT	19.29 ±1.46 ^c
<i>Jasmine 85</i>	NaCl	3.58 ±0.26 ^a
<i>Jasmine 85</i>	Urea	17.75 ±0.31 ^b
<i>Jasmine 85</i>	DTT	33.20 ±4.53 ^c

4.3.2 Protein Solubility of Rice Flours

Protein solubility is known to be the proportion of nitrogen in a protein that is in the soluble state under specific conditions. It is affected by both environmental (ionic strength, type of solvent, pH and temperature) and processing conditions (Zayas, 1997). In most cases, amino acids composition and sequence, molecular weight and content of polar and nonpolar groups in amino acids influence protein solubility (Zayas, 1997). Significant information on the use of proteins and their functionality are obtained from knowledge on protein solubility.

Figure 4.1 (panel A) shows that *Jasmine 85* rice proteins were significantly more soluble than *Viwonor* rice proteins in all three buffers. This may be related to the better protein quality in *Jasmine 85* rice and not only higher protein content. Among the three buffers, the proteins from both *Jasmine 85* and *Viwonor* rice were least soluble in the saline buffer. The solubility markedly increased for both rice varieties in urea probably because there were more basic proteins. Addition of DTT to urea tremendously improved the solubility of proteins of both rice varieties. This is because the addition of the DTT

hydrolyzed the sulphur-sulphur groups and caused the release of more protein aggregates in both rice.

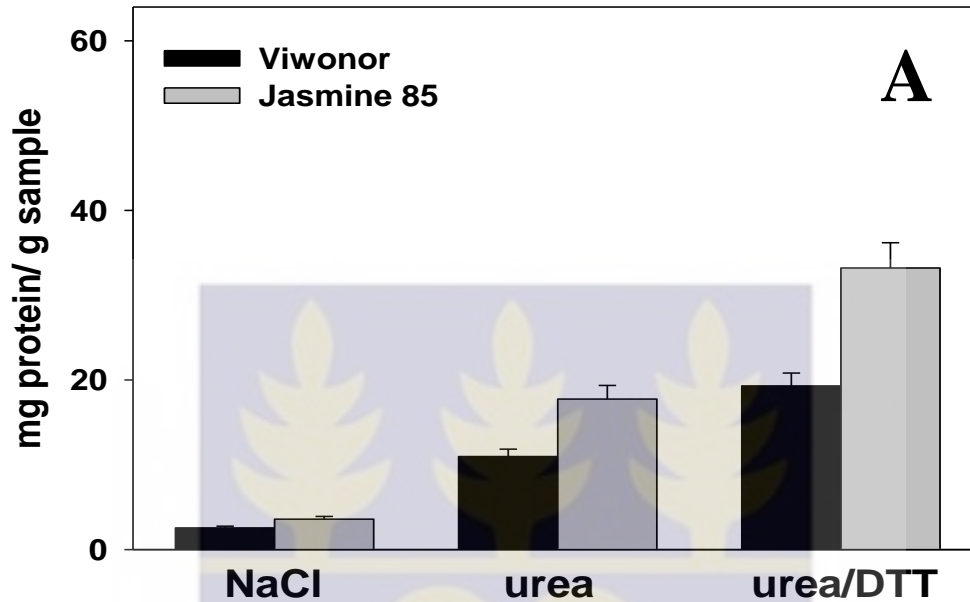


Fig 4. 1: Soluble proteins in saline buffer (NaCl), containing urea and urea/DTT, when indicated from rice flours (panel A)

4.3.3 Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) of Rice Proteins

Gel electrophoresis (SDS-PAGE) is the most widely used method for separation and analysis of proteins and nucleic acids. The technique allows the testing of the formation of disulfide-linked soluble aggregates in the presence or absence of 2-mercaptoethanol. In this assay, an electric current is passed through a gel from one electrode to the other thus movement of charged particles in an electric field toward an electrode of opposite charge.

SDS-PAGE tracings of extracts from rice samples (Fig. 4.2) showed no differences in the composition of the proteins. Protein bands were similar for both *Viwonor* and *Jasmine 85* rice varieties (Fig 4.2). Figure 4.2 confirms the observed variability in the solubility of proteins in the three different buffers. The saline buffer extracted mainly lower

molecular weight proteins which are mostly albumins. The urea buffer extracted more protein bands than the saline in both rice cultivars. The figure shows that a total of 7 bands of both high and low molecular weight proteins were extracted by the urea buffer. When DTT was added to urea as the third extracting buffer, an extra protein band (MWT 30KDa) was visible in both *Viwonor* and *Jasmine 85*. The higher amount of extractable proteins resulting in a deeper intensity of protein bands on the gel in *Jasmine 85* rice can be related to the higher quality of protein in this rice variety, and confirms the results from solubility assays. The rice proteins extracted were generally of low molecular weight ranging from 14.4 to 40 KDa.

Rice Flours

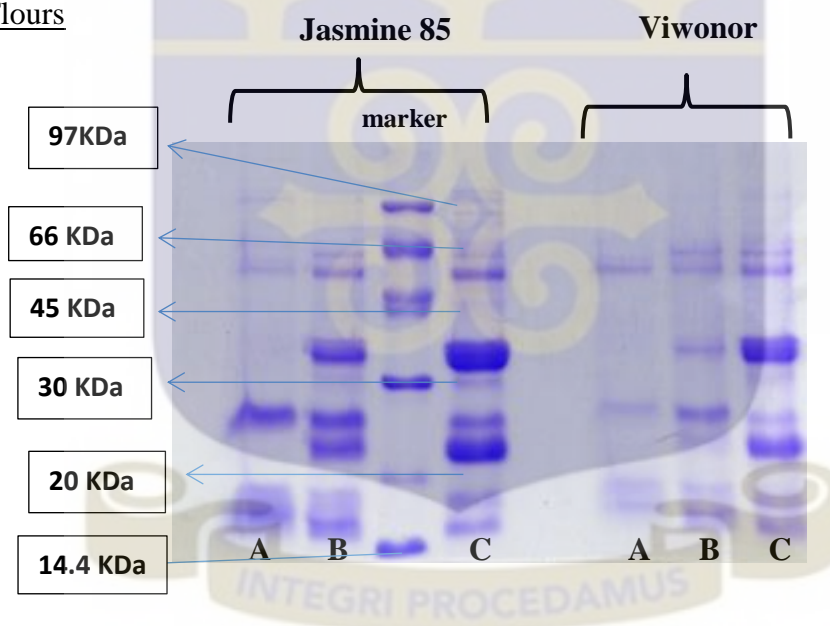


Fig 4.2: SDS-PAGE of proteins extracted from rice samples (*Jasmine 85* and *Viwonor*). Proteins were solubilized in: saline buffer (A); buffer in the presence of 6 M urea (B); buffer in the presence of 6 M urea and 10 mM DTT (C). Samples were treated in the presence of β -mercaptoethanol prior to protein separation.

4.3.4 In vitro Digestibility of Proteins (pepsin and pancreatin) of Rice Flours

Protein digestibility is one of the most important factors in determining protein quality of raw materials and their products (FAO, 1985). In vitro protein digestibility provides information on how a particular protein has been efficiently digested and identifies changes in protein quality (Damodaran, 1996; Day and Swanson, 2013).

The protein hydrolyses trends show that *Viwonor* rice tendered to have lower digestibility than *Jasmine 85* rice to pepsin but in particular the pancreatin hydrolyses (Fig. 4.3).

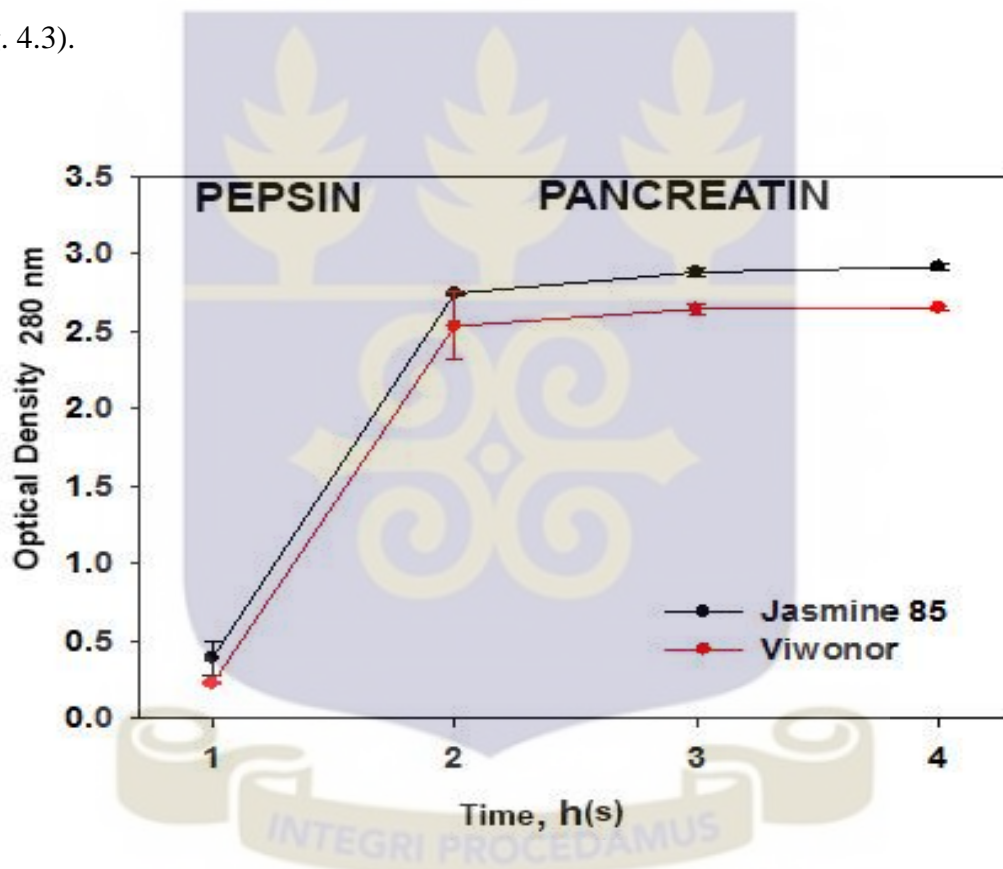


Fig 4.3: In vitro Pepsin and Pancreatin Digestion of Rice Flours

4.3.5 Accessible Thiols of Rice Flours

The nutritional value of protein-containing food is not only dependent on the quantity and digestibility of the protein, but also on the amino acid composition and availability of essential amino acids (Day and Swanson, 2013). Thiol–disulfide exchange reactions are major contributors to the formation of a covalently-linked protein network in many

foods, where disulfides represent the most “natural” type of inter-protein covalent bond (Iametti *et al.*, 2013). In this frame, thiol-disulfide exchange reactions occur as a function of the accessibility of the involved thiols, which in turn depends on structural features of the proteins.

The data in figure 4.4 show that the accessible thiols were measured in both *Viwonor* and *Jasmine 85* rice are the same in the presence/absence of denaturant. This suggests that the overall protein organizations of the rice samples are similar and loose in these matrices.

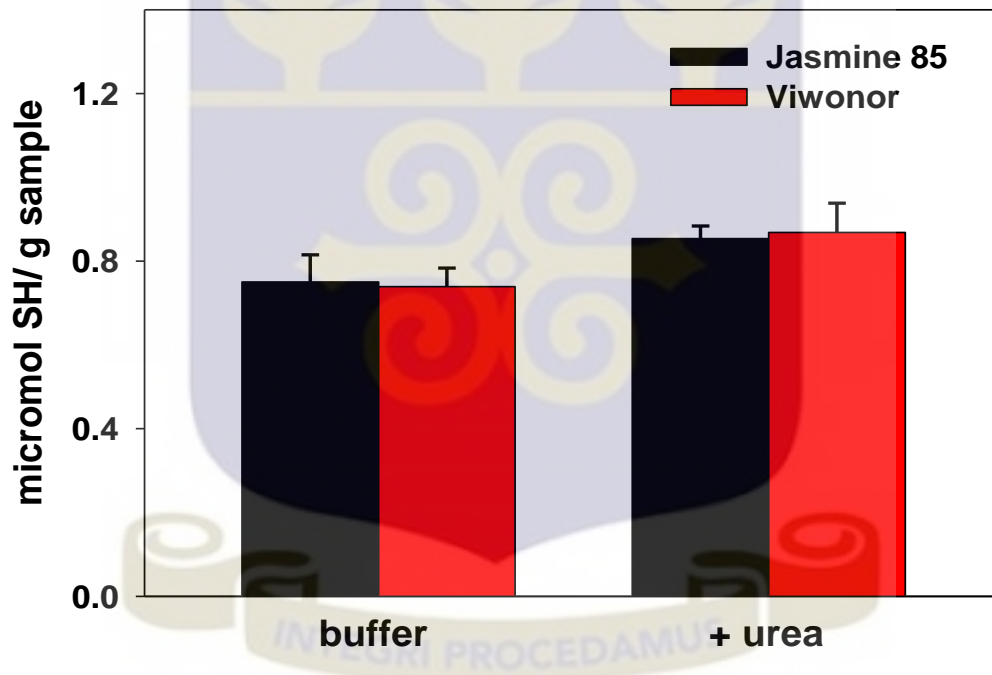


Fig 4.4: Accessible Thiols of Rice Flours

4.4 Chemical Properties of Sprouted Cowpea Flours

4.4.1 Moisture Content (%) of Sprouted Cowpea Flours

Sprouting of cowpea seeds significantly increased its moisture contents. The highest moisture content was recorded in I day sprouted cowpea. This suggests that the germination procedure promoted the increase in hydrophilic compounds (sugars,

dextrins, peptides, amino acids etc.) responsible for the higher water affinity which resulted in the higher moisture content (Moongngarm and Saetung, 2010). However, there was no significant difference in moisture with respect to 1 and 3 day germinated cowpea seeds.

Table 4.8: Moisture Content (%) of Sprouted Cowpea Flours

Sample	Moisture Content (%)
Cowpea 0	5.42 ±0.05 ^a
Cowpea 1	8.40 ±0.08 ^c
Cowpea 2	7.06 ±0.21 ^b
Cowpea 3	8.00 ±0.04 ^c

4.4.2 Protein Content Sprouted Cowpea Flours

Protein contents in sprouted cowpea flour samples were assayed with saline, urea and Dithiothreitol (DTT) buffer. The saline buffer extracted the salt soluble proteins of the cowpea samples while the urea buffer which is strongly basic solubilized the basic proteins. Extraction of proteins with the organic solute urea denatured more of the proteins in the cowpea flour samples. A further increase in protein content was measured with the DTT buffer in both rice samples (Table 4.9). The addition of the DTT to the buffer allowed the reduction of disulphide linkages and increased the quantity of proteins extracted. Protein quantities of cowpea were observed to have decreased with increasing sprouting time across all buffers. This may be to the fact the storage proteins in the cowpea (mainly globulins) may have metabolized into the growth of plant during sprouting.

Table 4.9: Protein Content of Sprouted Cowpea Flours

Sample	Buffer	Protein Content mg/g sample
Cowpea 0	NaCl	81.61 ±5.51 ^a
Cowpea 0	Urea	150.36 ±26.13 ^c
Cowpea 0	DTT	118.47 ±18.45 ^b
Cowpea 1	NaCl	59.78 ±3.59 ^a
Cowpea 1	Urea	61.95 ±5.64 ^a
Cowpea 1	DTT	67.93 ±6.92 ^b
Cowpea 2	NaCl	53.89 ±0.64 ^a
Cowpea 2	Urea	98.55 ±10.25 ^b
Cowpea 2	DTT	52.71 ±2.31 ^a
Cowpea 3	NaCl	38.13 ±1.67 ^a
Cowpea 3	Urea	73.55 ±3.59 ^c
Cowpea 3	DTT	45.10 ±3.84 ^b

Note: Cowpea 0= unsprouted cowpea, Cowpea 1=1 day sprouted cowpea,

Cowpea 2=2 day sprouted cowpea and Cowpea 3=3 day sprouted cowpea

4.4.3 Solubility of Sprouted Cowpea Proteins

Cowpeas were sprouted for 1, 2 and 3 days and used to make breakfast cereal formulations with rice flour (*Vivonor* and *Jasmine 85*). The solubility properties of the cowpea flour were determined in three different buffer solutions. Figure 4.5 (panel B) revealed that the solubility differed in the three buffer solutions. The solubility of cowpea proteins in saline were generally low but improved when they were solubilized in urea. The solubility of the cowpea proteins further increased in the buffer containing both urea and dithiothreitol (DTT). In all three buffer solutions, the solubility of proteins from unsprouted cowpea was significantly higher than proteins from sprouted cowpeas. The protein solubility trends showed that increasing germination time significantly decreased amount of solubilized proteins in all buffers.

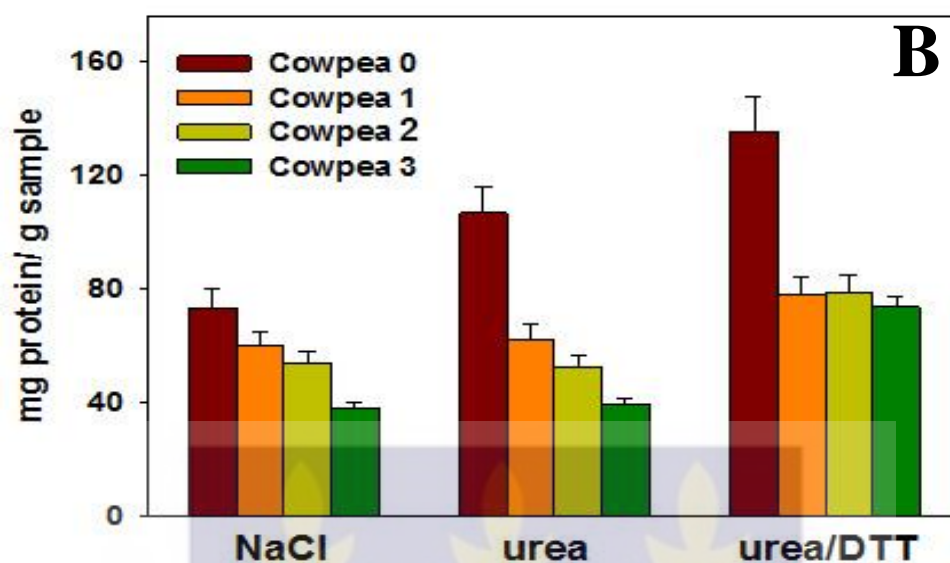


Fig 4.5: Soluble proteins in saline buffer (NaCl), containing urea and urea/DTT, when indicated from cowpea flours at different germination times (panel B).

4.4.4 SDS-PAGE of Sprouted Cowpea Proteins

Figure 4.6 and Figure 4.7 show the electrophoretic patterns of protein extracts of cowpeas at different germination times in three different buffers. Since this was a qualitative determination of proteins, there was no significant variability in the number of protein bands for each germination day and by the different buffer solutions. For all three extraction buffers the major protein bands were within 45 and 66 KDa which are mainly globulins (Franklin *et al.*, 1957; Richard *et al.*, 1981)). Generally, cowpea proteins of higher molecular weight were mostly extracted in all buffers.

4.4.5 The Role of Saline, Urea and DTT Buffers in Extracting Proteins (SDS-PAGE)

More protein bands were extracted by the alkaline buffers (urea and urea/DTT) in all extrudates irrespective of sprouting treatments. Based on the intensity of the bands, the presence of DTT in urea buffer enabled more proteins to be extracted. This may be due to the many disulphide linkages cowpea globulins (Cabrera-Chávez *et al.*, 2012; Bonomi

et al., 2012; Mariotti *et al.*, 2011) that were broken by the DTT and consequently facilitated the protein extraction. Cowpea proteins are rich in lysine, arginine and leucine which are basic amino acids (Iqbal *et al.*, 2006). The extraction of more proteins in the urea buffer may be due to the presence of high amount of the basic amino acids (lysine, leucine and arginine) in the cowpea (House *et al.*, 1987

Cowpea Flour

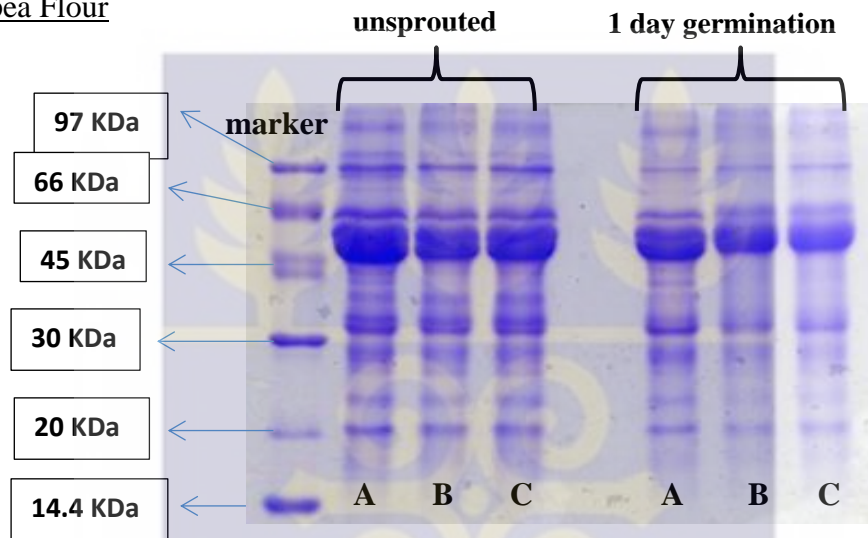


Fig 4.6: SDS-PAGE of proteins extracted from cowpea samples (unsprouted and after 1 day germination). Proteins were solubilized in: saline buffer (A); buffer in the presence of 6 M urea (B); buffer in the presence of 6 M urea and 10 mM DTT (C). Samples were treated in the presence of β -mercaptoethanol prior to protein separation



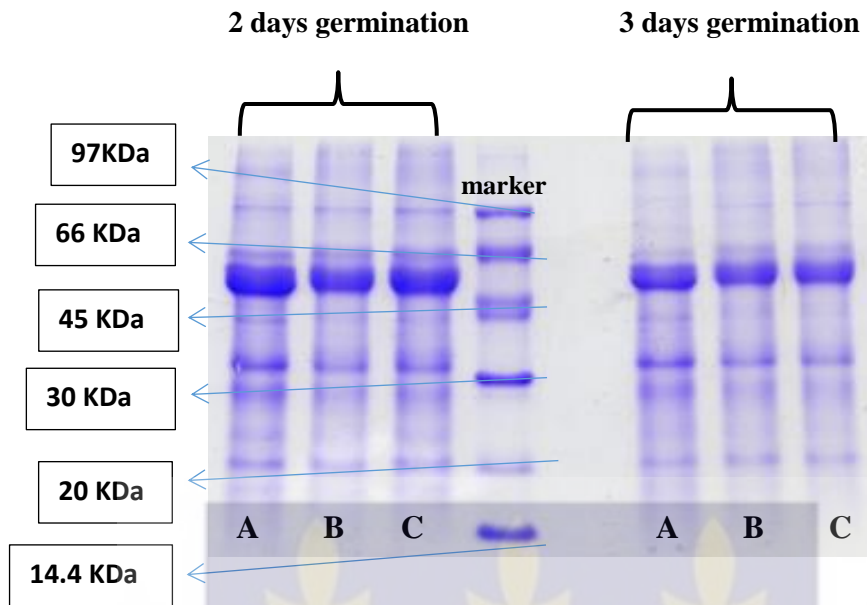


Fig 4.7: SDS-PAGE of proteins extracted from cowpea samples (respectively after 2 and 3 days germination). Proteins were solubilized in: saline buffer (A); buffer in the presence of 6 M urea (B); buffer in the presence of 6 M urea and 10 mM DTT (C). Samples were treated in the presence of β -mercaptoethanol prior to protein separation.

4.4.6 In vitro Digestibility of Proteins (pepsin and pancreatin) of Sprouted Cowpea Flours

Figure 4.8 show the rate of pepsin and pancreatin digestion of cowpea proteins. Cowpeas that were sprouted for three days were far more digestible by pepsin and pancreatin enzymes than cowpea than were not sprouted for that long. Long term sprouting may either have removed protease inhibitors or may have transformed the proteins to be more susceptible to protease digestion. Protein hydrolysis occurs during sprouting of cowpea which results in the availability of more proteins for enzyme digestion.

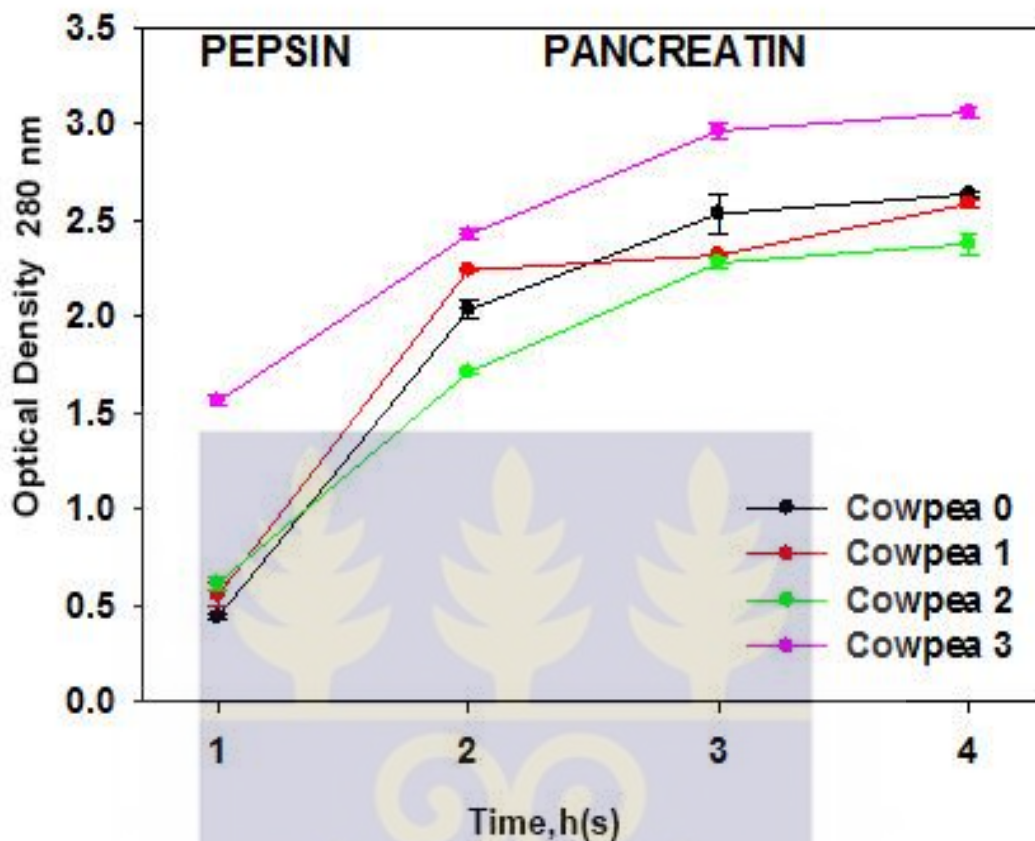


Fig 4.8: In vitro Pepsin and Pancreatin Digestion of Sprouted Cowpea Flours.

4.4.7 Accessible Thiols of Sprouted Cowpea Flours

The degree of structural “stiffness” of the protein network in individual samples was evaluated through thiol accessibility studies in the presence/absence of urea. These studies were carried out on protein suspensions (Iametti *et al.*, 2006; Mariotti *et al.* 2008) and the measurements provided two separate parameters, namely the total content in reactive thiol (measured under denaturing conditions on both the soluble and insoluble fraction) and the increment in thiol accessibility due to denaturation.

Increasing germination time of cowpea seeds significantly decreased ($p < 0.05$) the amount of accessible thiols with or without the denaturant (Fig. 4.9). This results show the variability in the structure of proteins in the cowpea samples. The measurement of

high accessible thiols in the unsprouted cowpea seeds suggests the presence of more thiols for the formation of more covalently linked protein network from the thiols-disulphide exchange reaction.

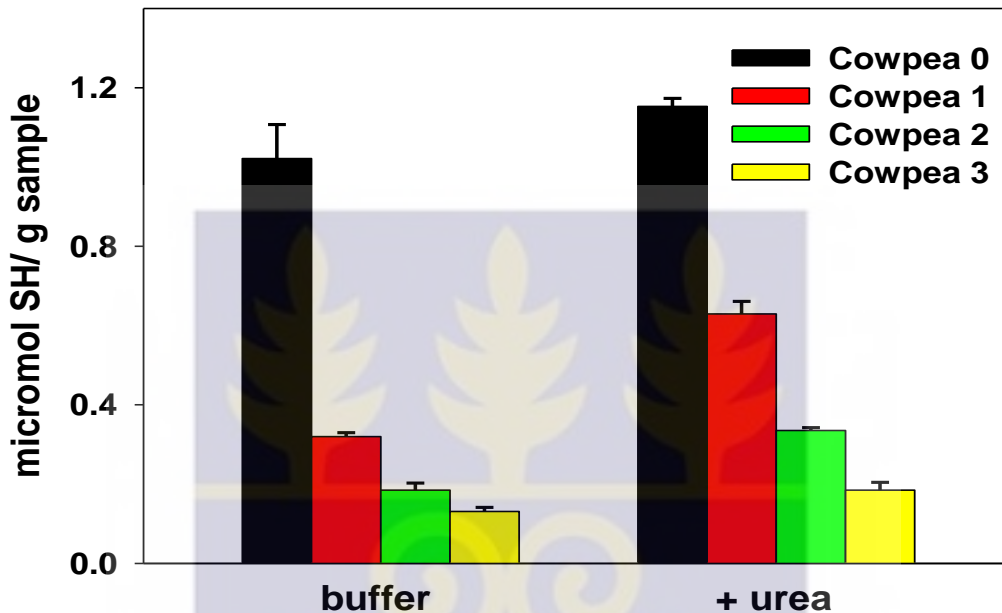


Fig 4.9: Accessible Thiols of Sprouted Cowpea Flours

4.4.8 Physical Property of Cowpea Flours

4.4.8.1 Colour of Cowpea Flours

Lightness index (L^*) of the cowpea flours significantly decreased ($p < 0.05$) with increasing sprouting time. However, both redness (a^*) and yellowness (b^*) indices tended to have increased with increasing sprouting time of cowpea. The Total Colour Difference (ΔE^*) of the cowpea flours was also significantly different with the least colour difference occurring in the longest sprouting time of cowpea. Cowpea sprouting time strongly affected the hue angle (h^*) of the flours (Table 4.10). The highest hue angle (h^*) of the cowpea flours was measured in the 1 day sprouted cowpea.

Table 4.10: Colour Profile of Cowpea flours

Sample	Lightness Index (L*)	Redness Index (a*)	Yellowness Index (b*)	Total Difference (ΔE^*)	Colour Hue angle h*
Cowpea 0	87.1 \pm 0.14 ^d	-0.89 \pm 0.01 ^a	15.33 \pm 0.84 ^a	1.21 \pm 0.56 ^b	86.70 \pm 0.86 ^b
Cowpea 1	80.61 \pm 0.37 ^c	0.58 \pm 0.13 ^b	17.67 \pm 1.82 ^b	3.62 \pm 0.47 ^d	88.10 \pm 0.53 ^c
Cowpea 2	78.25 \pm 0.35 ^b	1.29 \pm 0.03 ^c	23.28 \pm 1.26 ^c	1.85 \pm 0.09 ^c	86.83 \pm 0.11 ^b
Cowpea 3	73.90 \pm 0.13 ^a	2.05 \pm 0.15 ^d	25.79 \pm 0.23 ^d	0.42 \pm 0.16 ^a	85.44 \pm 0.42 ^a

4.5 Formulation of rice - sprouted cowpea flours for the production of breakfast cereals

The Central Composite Rotatable Design (CCRD) resulted in 13 experiments consisting of 8 experimental runs with 5 centre points. 13 flour formulations were generated and extruded. The flour formulations have been presented in Table 4.11.

Table 4.11: 13 Flour Formulations and their Composition

Sample	Code	Composition
1	Formulation 1	30% unsprouted cowpea + 70% <i>Viwonor</i> rice
2	Formulation 2	30% 3 day sprouted cowpea + 70% <i>Viwonor</i> rice
3	Formulation 3	70% unsprouted cowpea + 30% <i>Viwonor</i> rice
4	Formulation 4	70% 3 day sprouted cowpea + 30% <i>Viwonor</i> rice
5	Formulation 5	50% unsprouted cowpea + 50% <i>Viwonor</i> rice
6	Formulation 6	50% 3 day sprouted cowpea + 50% <i>Viwonor</i> rice
7	Formulation 7	22% 2 day sprouted cowpea + 78% <i>Viwonor</i> rice
8	Formulation 8	78% 2 day sprouted cowpea + 22% <i>Viwonor</i> rice
9	Formulation 9	30% 2 day sprouted cowpea + 70% <i>Viwonor</i> rice
10	Formulation 10	50% 2 day sprouted cowpea + 50% <i>Viwonor</i> rice
11	Formulation 11	50% 2 day sprouted cowpea + 50% <i>Viwonor</i> rice
12	Formulation 12	50% 2 day sprouted cowpea + 50% <i>Viwonor</i> rice
13	Formulation 13	50% 2 day sprouted cowpea + 50% <i>Viwonor</i> rice

4.5.1 Results of Consumer Test Analysis

Analysis of variance (ANOVA) was performed on the results of the consumer preference test on the extrudates. Table 4.12 shows the mean acceptability score of the extrudates. Cowpea ratio and sprouting time significantly affected the overall consumer acceptability ($p < 0.05$) of the extrudates. Extrudate 7 which was produced from the minimum level of cowpea substitution (22%) with *Viwonor* rice was most acceptable. This was followed by extrudate 1 and 2 with 30% unsprouted cowpea +70% *Viwonor* rice and 30% 3 day sprouted cowpea + 70 *Viwonor* rice respectively (Table 4.12). The least liked extrudate was extrudate 8 (with 78% 2 day sprouted cowpea + 22% *Viwonor* rice). This shows the high consumer dislike for extrudates with higher cowpea ratio.

Table 4.12: Mean Acceptability Score for Extrudates

Sample	Code	Cowpea Sprouting Time (days)	Cowpea ratio (%)	Estimated Mean	Rank Order
Extrudate 1	1	0	30	6.196	2nd
Extrudate 2	2	3	30	5.935	3rd
Extrudate 3	3	0	70	5.152	7 th
Extrudate 4	4	3	70	5.065	9 th
Extrudate 5	5	0	50	5.261	6 th
Extrudate 6	6	3	50	5.152	7 th
Extrudate 7	7	2	22	6.761	1st
Extrudate 8	8	2	78	3.696	13 th
Extrudate 9	9	2	30	3.969	12 th
Extrudate 10	10	2	50	4.239	10 th
Extrudate 11	11	2	50	5.652	4 th
Extrudate 12	12	2	50	5.630	5 th
Extrudate 13	13	2	50	4.00	11 th

4.5.2 Selection and Production of Best 6 Extrudates

Extrudates made with the minimum level of cowpea substitution (22%) with *Viwonor* rice was most acceptable by consumers as a breakfast cereal. The best three extrudates (Extrudates 7, 1 and 2) were selected from the mean acceptability scores generated in Table 4.12. Similar products (of 30% cowpea + 70% rice) were produced from the relatively higher grade rice (*Jasmine 85*) and were comparable. A total of six extrudates were obtained for further analysis.

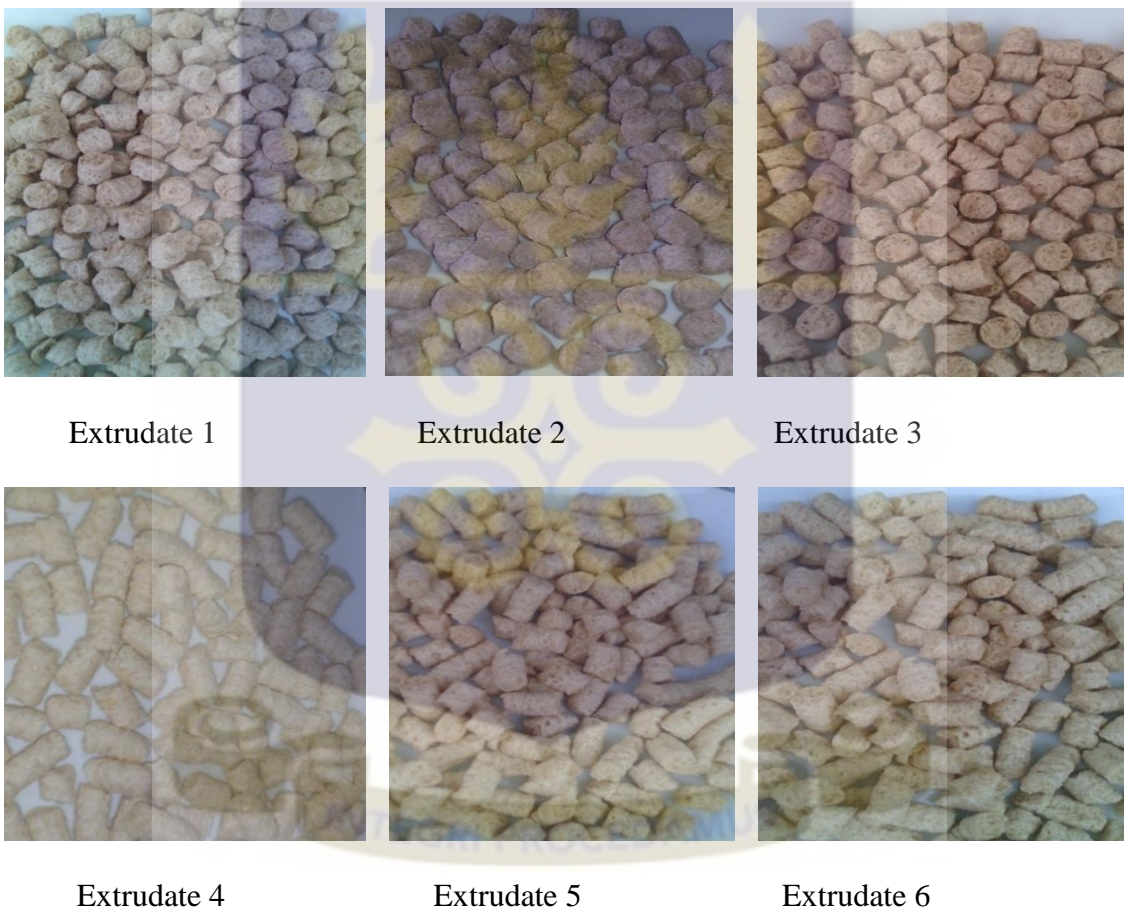


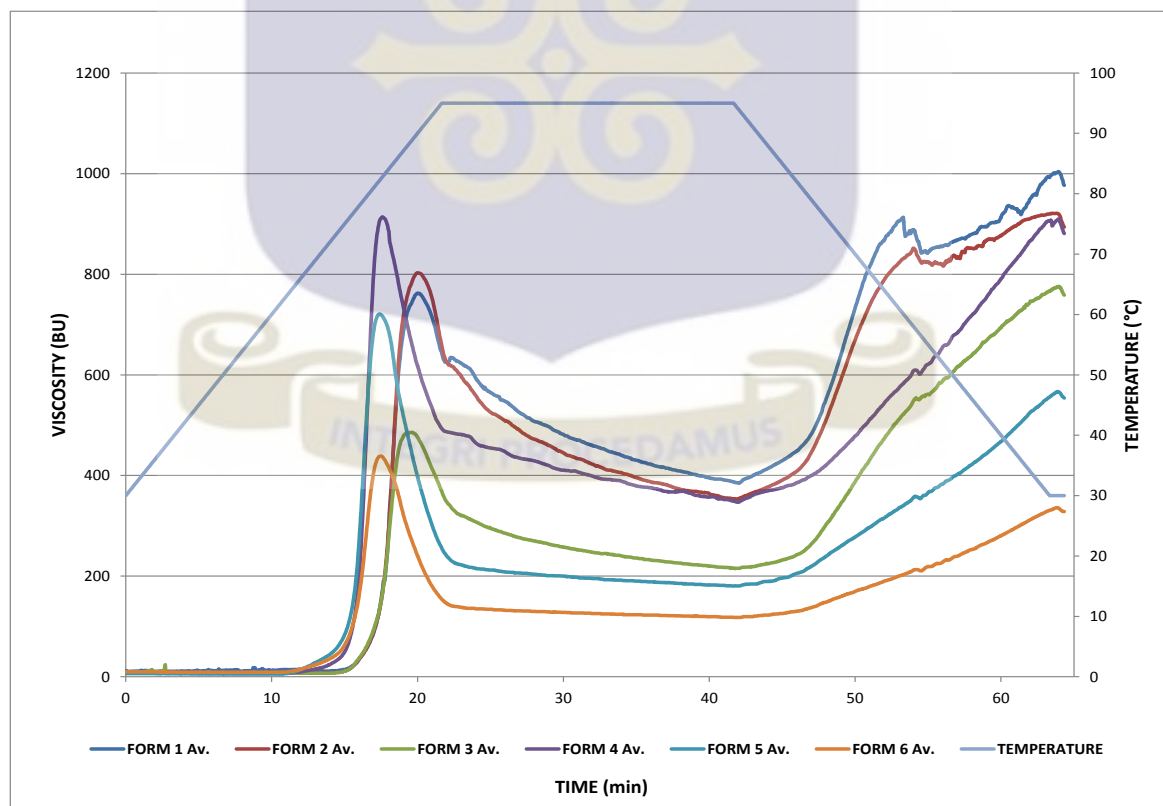
Plate 4.1: Images of Best 6 Extrudates

Table 4.13: Final 6 Extrudates and their Composition

Sample	Composition
Extrudate 1	30% unsprouted cowpea+70% <i>Viwonor</i> rice
Extrudate 2	30% 2 day sprouted cowpea+70% <i>Viwonor</i> rice
Extrudate 3	30% 3 day sprouted cowpea+70% <i>Viwonor</i> rice
Extrudate 4	30% unsprouted cowpea +70% <i>Jasmine 85</i> rice
Extrudate 5	30% 2 day sprouted cowpea +70% <i>Jasmine 85</i> rice
Extrudate 6	30% 3 day sprouted cowpea +70% <i>Jasmine 85</i> rice

4.5.3 Pasting Properties of Flour Formulations

Pasting behaviour of flour samples is the phenomenon following gelatinization of starch, granules swelling, exudation of molecular components from the granules and total disruption of the granules (Atwell *et al.*, 1998). Pasting property is an indication of starch behaviour upon cooking.

**Fig 4.10: Pasting Profile of flour formulations.**

Samples are identified as interpreted by the legend.

Figure 4.10 show the pasting profile of rice and cowpea flour formulations. Variations in flour formulations significantly affected ($p < 0.05$) pasting temperatures of the flours. In particular, the lowest pasting temperatures were associated with *Jasmine 85 rice* - based flour samples (formulations 4, 5 and 6), whereas the highest were measured in the *Viwonor rice* - based flours (formulations 1, 2 and 3). This could be attributed to the stronger bond strength within the starch molecules as reported by Eliason and Karlson, (1983). Higher pasting temperatures are associated to stronger inter-granular bonds as can be observed in the case of *Viwonor rice* - based flours (Table 4.11) indicating the likely presence of stronger inter-granular within the granules. It has been established that more energy is required to break the bonds in amylose-rich starches and this corresponds to the high pasting temperatures recorded in the *Viwonor rice* (amylose-rich) based flours.

Gelatinization temperature (GT) has been shown to be dependent on the degree of cross-linking of the amylopectin as Shi and Seib, (1995) reported that higher levels of long chains of amylopectin in maize tended to increase GT. This suggests the possible presence higher levels of long chains of amylopectin within the *Viwonor rice*. GT depends on the presence and amount of damaged starch granules which increases swelling capacity. For the rice-cowpea formulations, gelatinization temperature is strictly dependent on the rice variety, making *Viwonor* variety more prone to this phenomenon. The significantly higher amylose was measured in *Viwonor* reflected corresponding higher gelatinization temperature in the *Viwonor rice* - based flours (Table 4.14).

Peak viscosities of the flour formulations were significantly influenced ($p < 0.05$) by the differences in rice and cowpea composition. The highest peak viscosity was measured in formulation 4 (*Jasmine 85* with unsprouted cowpea) at 915.0 BU, whereas formulations

3 and 6 showed the lowest viscosity peak (486 and 438.5 BU) respectively. The addition of germinated cowpea flour to the rice resulted in decrease in peak viscosities.

Table 4.14: Pasting Properties of Flour Formulations

Sample	Peak Viscosity (BU)	Breakdown (BU)	Setback (BU)	Final Viscosity (BU)	Pasting Temperature (°C)
Formulation 1	762.00	374.50	604.50	976.50	89.30
Formulation 2	802.50	449.00	566.50	893.50	89.15
Formulation 3	486.00	270.50	552.00	758.00	88.20
Formulation 4	915.00	566.00	557.00	881.00	82.80
Formulation 5	720.00	540.50	375.00	554.00	82.50
Formulation 6	438.50	320.50	213.00	328.50	82.85

Decrease in viscosities was observed during the holding period at 95°C (Fig. 4.10). This phenomenon is related to disruption of starch granules and leaching of amylose when flours are subjected to high temperature and mechanical shear. Dramatic decrease in viscosities was observed in all formulations at the holding period.

Viscosities increase due to the formation of gel structure indicating the tendency of the granules to re-associate or retrograde during cooling (Bonomi *et al.*, 2012). Retrogradation is the process during which gelatinized starch granules undergo rearrangements upon cooling and form a structure with a higher degree of crystallinity (Ottenhof and Farhat, 2004). Retrogradation is known to be faster in relatively larger particle sized granules compared to smaller ones due to the highly compacted nature of the smaller granules; it is therefore known to be faster in amylose than in amylopectin (Ottenhof and Farhat, 2004). This was observed when increased set back viscosities were measured in the amylose-rich *Viwonor* rice based formulations (Table 4.14). The

faster occurrence of retrogradation in amylose has been attributed to the linear structure of the inherent granules by Van Soest *et al.*, (1994). Conclusively, flours with lower starch amylose contents corresponded to higher peak viscosity, lower pasting temperature and greater resistance to retrogradation as suggested by (Pham Van *et al.*, 2006).

4.6 Physical Characterization of Rice – Cowpea Extrudates

Physical characteristics of an extruded product such as expansion, hardness, density and porosity are important parameters for functional characteristics and consumer acceptability of the products. (Tahvonen *et al.*, 1988). For the purpose of the study, bulk density, expansion ratios, colour, texture (hardness) and porosity (by image analysis) of the extrudates were assayed.

4.6.1 Expansion Ratio of Rice and Cowpea Extrudates

Expansion is one of the most important properties of extruded products obtained through high temperature, low moisture extrusion cooking (Patil *et al.*, 2007). Expansion promotes dehydration and the development of a desirable crispy texture on the final extruded product (Patil *et al.*, 2007).

Expansion ratio of extrudates was significantly affected ($p < 0.05$) by rice cultivar and germination time of cowpea. Extrudates made from *Jasmine 85* rice were characterized with higher expansion ratios whereas those of the *Viwonor* rice were lower. This suggests that the higher presence of amylose in the *Viwonor* rice retarded the expansion rates of the extrudates. It was observed that the expansion ratio of the extrudates was negatively influenced by rice grain hardness. This was seen as the *Viwonor rice* – based extrudates with higher hardness index measured relatively lower expansion ratios.

The trend showed that the addition of cowpea flour to the flour mixtures significantly decreased expansion ratios in all extrudates irrespective of the rice cultivar (Table 4.15). It is possible that the high presence of hydrolyzed proteins and starches in the germinated cowpea seeds retarded the expansion ratio of the extrudates. Specifically the hydrolyzed proteins and starches in the germinated cowpea seeds resulted in a more compact matrix which consequently inhibited the expansion of the extrudates.

Table 4.15: Bulk Densities and Expansion Ratio of Extrudates

Sample	Bulk Density g/cm ³	Expansion Ratio
Extrudate 1	0.08 ±0.00 ^a	3.23 ± 0.04 ^c
Extrudate 2	0.08 ±0.00 ^b	2.99 ± 0.02 ^b
Extrudate 3	0.11 ±0.00 ^c	2.73 ± 0.02 ^a
Extrudate 4	0.11 ±0.01 ^c	3.76 ± 0.02 ^c
Extrudate 5	0.10 ±0.01 ^b	3.65 ± 0.03 ^b
Extrudate 6	0.08 ±0.00 ^a	3.36 ± 0.01 ^a

4.6.2 Bulk Densities of Rice - Cowpea Extrudates

Bulk density is defined as the overall mass of the food material divided by the volume occupied by the food material (Singh and Heldman, 2014). It depends on the composition of the food ingredient and can be directly related to the free space volume of a package (Robertson, 2013). Table 4.15 shows the mean bulk densities of the extrudates. Rice cultivar had no effect ($p > 0.05$) on the bulk densities of all extrudates.

The bulk densities of rice-cowpea extrudates varied from 0.07 – 0.11 g/cm³ at the ratio of 30% cowpea and 70% rice (for both rice cultivars). Similarly, studies by Ravidran *et al.*, (2011) on rice-pea blend extrudates showed that bulk density values increased from 0.08

to 0.09 g/cm³ for guar gum; 0.08 g/cm³ for locust bean gum and 0.1 to 0.11 g/cm³ for ferrugreek gum with increasing levels of gum substitution.

Filli *et al.*, (2012) linked bulk density to expansion ratio in describing the degree of puffing in extrudates. Similarly, Fayose, (2007) described bulk density as a parameter that is controlled by degree of expansion. This confirms the observation that products that were the most puffed had higher bulk densities. *Viwonor* rice - based rice extrudates were generally less puffed than the *Jasmine 85* – based rice extrudates.

4.6.3 Colour Profile of Rice – Cowpea Extrudates

Appearance is one of the major factors consumers use in evaluating the quality of food product. The appearance of food is mostly determined by its surface colour and colour of food is the first quality parameter evaluated by consumers (Pathare *et al.*, 2013). Colour is basically an appearance property attributed to the spectral distribution of light and is related to the illuminant, the object to which the colour is ascribed and the observer's eye (Jha, 2010).

4.6.3 (1) Lightness index (L*) of the Extrudates

The lightness index (L*) of the extrudates was significantly affected ($p < 0.05$) by cultivar of rice. The natural reddish colour of the *Viwonor* rice resulted in a lower lightness index among the *Viwonor* rice –based extrudates (Fig. 4.11). On the other hand, higher lightness indices (L*) were observed for the *Jasmine 85* –based extrudates. This clearly shows the influence of the colour of the starting material on the final product.

The addition of cowpea flour with increased sprouting time significantly reduced the lightness (L*) of the extrudates irrespective of the rice cultivar. Specifically, *Jasmine 85* - based extrudates were characterized by higher colour lightness than *Viwonor* - based

ones. The decrease in lightness colour of extrudates could be due to the non-enzymatic browning reaction that occurred during the germination process.

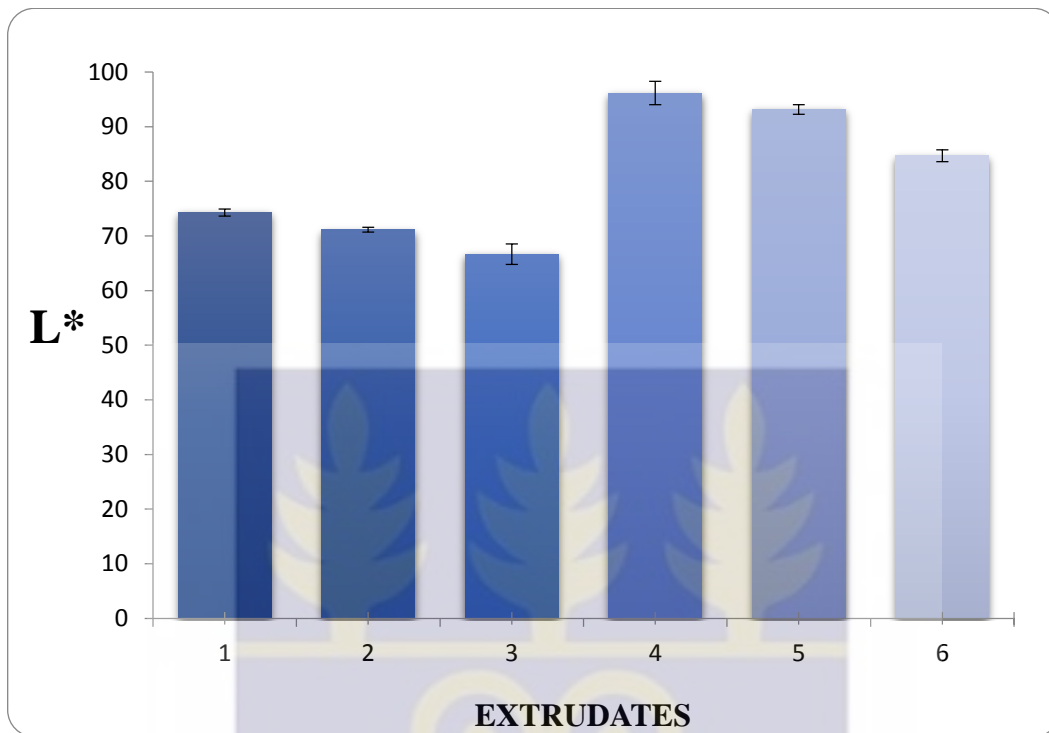


Fig 4.11: Colour Profile of Extrudates - Lightness Index (L*)

4.6.3 (2) Redness Index (a*) of the Extrudates

The cultivar of rice significantly affected the redness (a*) index of the extrudates. Higher redness was measured among the *Viwonor*-based extrudates (Fig. 4.12). Extrudates made from *Viwonor* rice recorded significantly higher redness index whereas those from *Jasmine 85* rice were lower. The influence of the rice cultivar on the colour of the extrudates was expected since both rice samples had different redness indices.

Increasing sprouting time imparted significant increase in redness of the *Viwonor* - based extrudates. Specifically, *Viwonor* - based extrudates showed higher redness index (a*), with the highest value being recorded in extrudate 3 (with 3 day sprouted cowpea flour) (Fig. 4.12). The *Jasmine 85* - based extrudates were associated with very low redness

indices (a^*) although the addition of sprouted cowpea flour improved the redness of its extrudates.

Extrusion cooking of sprouted cowpea flour based products significantly increased ($p < 0.05$) the redness (a^*) values among the *Viwonor* - based extrudates; suggesting the likely occurrence of Maillard reaction between the amino groups and the sugars within the rice-cowpea flour during sprouting and extrusion cooking.

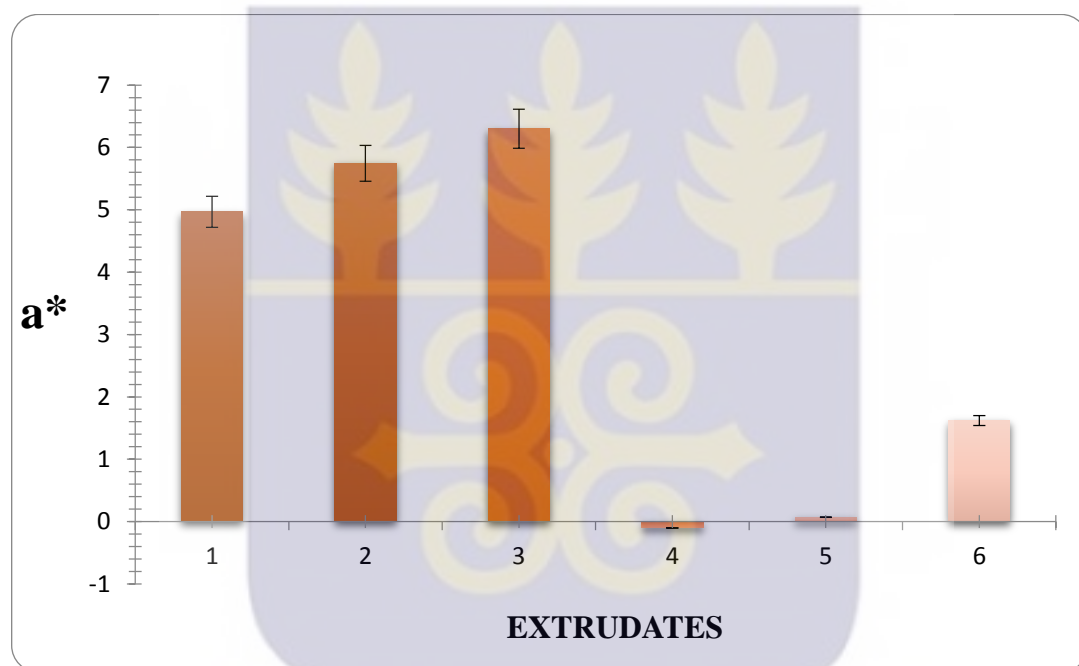


Fig 4.12: Colour Profile of Extrudates- Redness Index (a^*)

4.6.3 (3) Yellowness Index (b^*) of Extrudates

Rice cultivar significantly affected the yellowness indices of the extrudates. *Viwonor* rice - based extrudates show higher yellowness index (b^*) values as compared to those of the *Jasmine 85* rice ones. Increasing sprouting time significantly increased ($p < 0.05$) the yellowness (b^*) index of the extrudates irrespective of the rice cultivar. The tendency of a^* and b^* indices increasing with germination time indicates the occurrence of browning (non-enzymatic) in the extruded samples. Nutritional statuses of foods resulting from

Maillard reactions are vital in foods intended for nutritional purposes (Fukui *et al.*, 1993). The formation of some brown compounds (pigments) called melanoids resulting from maillard reactions during the germination of the cowpea seeds became evident as redness (a^*) and yellowness (b^*) colour intensities of the extrudates increased (Singh *et al.*, 2007).

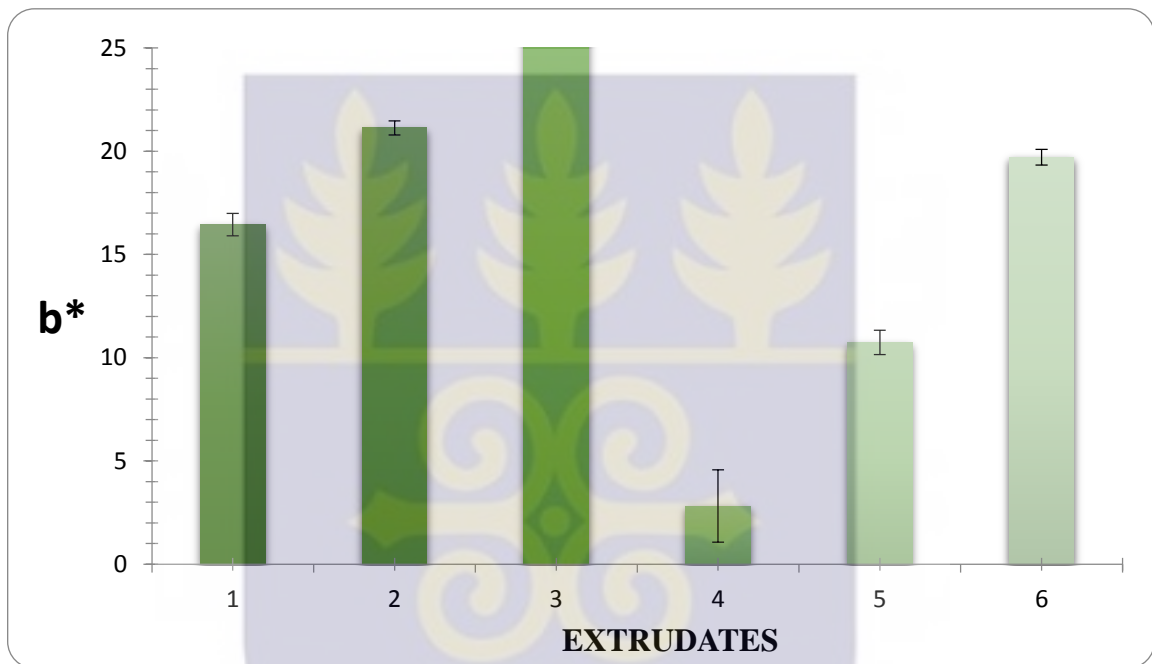


Fig 4.13: Colour Profile of Extrudates-Yellowness Index (b^*)

Assessment of color is more than a numeric expression. Usually it's an assessment of the color difference (delta) from a known standard. Total colour difference (ΔE^*) is defined as the difference between two colors in an L^* a^* b^* color space. The effects of rice variety and sprouting time of cowpea on the total colour difference of extrudates have been presented in Table 4.16. The ANOVA analysis showed a statistically significant influence of rice variety ($p < 0.05$) on total colour difference (ΔE^*) on extrudates. The total colour difference (ΔE^*) of extrudates made from *Jasmine 85* rice were generally

higher. Extrudates containing cowpea seeds that were sprouted for 2 days measured the least ΔE^* irrespective of the rice variety

Table 4.16: Colour Indices of Extrudates

Sample	Lightness Index (L*)	Redness Index (a*)	Yellowness Index (b*)	Total Colour Difference (ΔE^*)	Hue angle h*
Extrudate 1	74.38 \pm 0.62	4.97 \pm 0.16	16.44 \pm 0.54	0.77 \pm 0.19	73.27 0.11
Extrudate 2	71.14 \pm 0.46	5.75 \pm 0.17	21.13 \pm 0.34	0.52 \pm 0.29	74.95 0.32
Extrudate 3	66.65 \pm 1.88	6.30 \pm 0.25	25.98 \pm 0.33	0.87 \pm 0.08	76.39 0.44
Extrudate 4	96.15 \pm 2.13	-0.10 \pm 0.12	2.81 \pm 1.75	2.35 \pm 0.57	88.43 0.86
Extrudate 5	93.15 \pm 0.86	0.07 \pm 0.11	10.74 \pm 0.59	0.78 \pm 0.18	87.30 3.66
Extrudate 6	84.68 \pm 1.07	1.62 \pm 0.12	19.71 \pm 0.38	0.81 \pm 0.17	85.42 0.06

Hue is basically the major parameter defining the overall colour or appearance of a product. The hue angle (h*) of a given colour is its closest match within the range of pure or saturated colours.

The ANOVA analysis of hue angle (h*) of extrudates showed the significant effect of both rice cultivar and sprouting time of cowpea on extrudates. Significantly lower hue angle (h*) values were measured among the *Viwonor* rice – based extrudates. Increasing sprouting duration of cowpea significantly increased ($p < 0.05$) the hue angle (h*) of *Viwonor* rice– based extrudates while it decreased extrudates prepared from *Jasmine 85* rice.

Conclusively, *Viwonor* - based extrudates (extrudates 1, 2 and 3) measured higher redness a* and yellowness b* indices with lower lightness compared with *Jasmine 85* - based ones. The formation of compounds resulting from maillard reactions during sprouting positively influenced the redness and yellowness indices in all extrudates.

4.6.4 Texture Profile Analysis

Texture is one of the principle quality attribute used in the fresh and processed food industries to assess product quality and acceptability (Chen and Opara, 2013). Texture, as a sensory attribute can dominate the quality of a product; and it can be measured by objective (instrumental) and descriptive sensory (subjective or intrinsic) means (Paula and Conti-Silva, 2014). Food texture is defined as the rheological and structural attributes of the product perceptible by means of mechanical, tactile, visual and auditory receptors (Lawless and Heymann, 1998). It is used along the food chain to monitor and control quality.

Hardness is defined as the maximum force that occurs at any time during the first compression cycle (Simewing, 1999). Hardness of the extrudates was significantly influenced ($P < 0.05$) by rice cultivar. Specifically, *Viwonor* rice - based extrudates (extrudates 1, 2 and 3) significantly showed higher values of hardness (force) thus suggesting the presence of a more compact matrix.

Lower hardness values were measured among the *Jasmine 85* - based extrudates (extrudates 4, 5 and 6) indicating the presence of less compact matrix within the *Jasmine 85* - based extrudates.

Increasing sprouting time significantly increased the hardness index of the extrudates irrespective of the rice cultivar. It is possible that the hydrolyzed starches and proteins in the cowpea during germination acted as binders and caused the increase in hardness of the extrudates.

The expansion ratios of the extrudates was inversely correlated with its texture (hardness) profile. The relatively harder extrudates (extrudates produced from the *Viwonor* rice) measured lower expansion ratios and bulk densities whereas The *Jasmine*

85 – based extrudates with lower hardness index were characterized by higher expansion ratios and bulk densities.

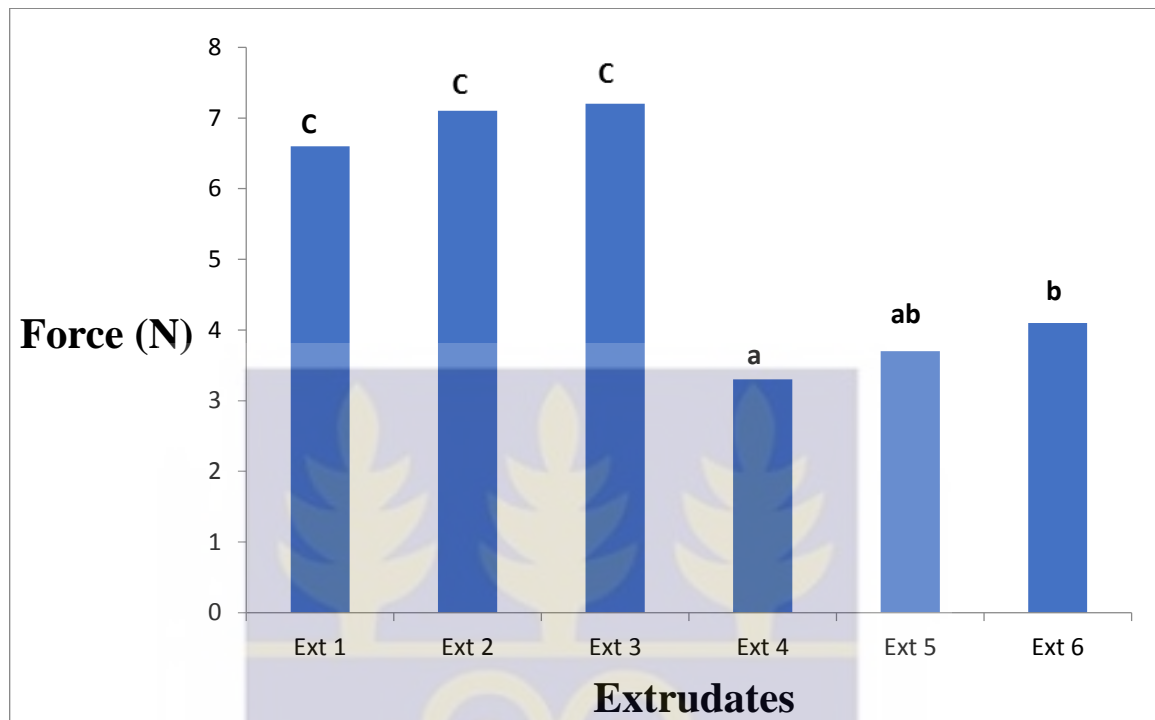


Fig 4.14: Texture Profile of Extrudates

Rheology is related to the deformation, disintegration and flow of the food when force is applied (Bourne, 2002). In this study, the force needed to puncture 50% of each extrudate was divided by the height of each sample (Fig. 4.14). The results of the measured force (N) are in accordance with the expansion index of the extrudates in that the most puffed extrudates were less hard (Fig. 4.15). As the expansion index of the *Jasmine 85* rice – based extrudates increased, the force required in puncturing them decreased. This suggests a less compact and more porous matrix in the *Jasmine 85* rice - based extrudates.

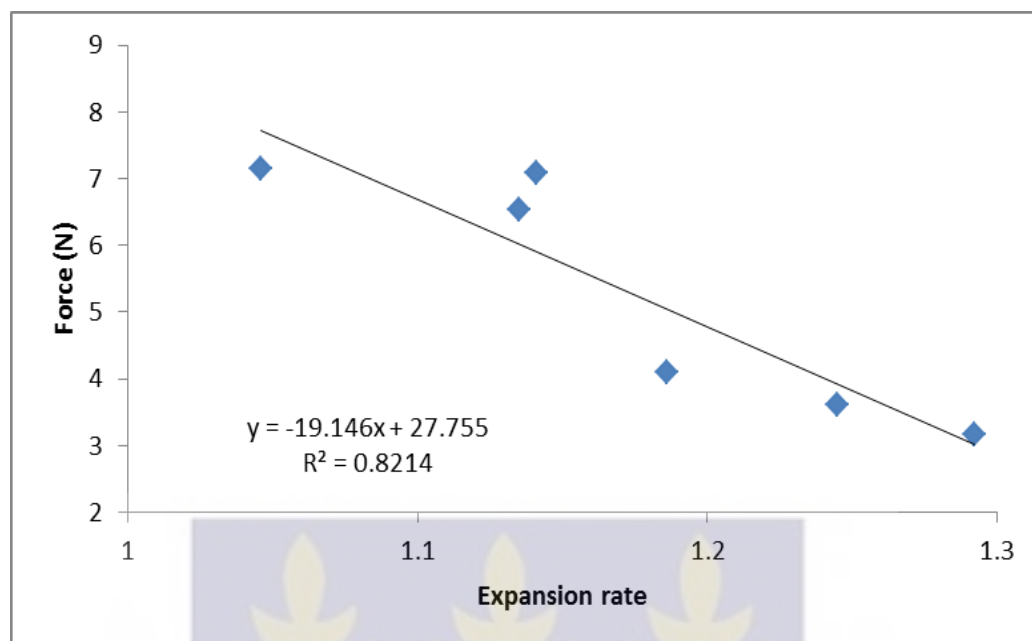


Fig 4.15: Correlation between expansion rate (X axis) and puncture force (Y axis)

4.6.5 Porosity of Rice – Cowpea Extrudates (Image Analysis)

Image processing techniques has been adopted to characterize the internal structure of expanded products (Tan *et al.*, 1994). Essentially, image analysis allows the quantification of the distribution of porosity in extrudate sections according to their size into three classes (encoded by 1 to 3). In this classification, class 1, includes the smallest bubbles, class 2 is medium bubbles and class 3 is large bubbles. The quality of extrudates depends on the crumb structure. The crumb structure is dependent on the uniform distribution of the bubbles. Extrudates like any other expanded products should have uniformly distributed small bubble size. The image analysis was done to study the crumb structure of the extrudates.

Analysis of variance (ANOVA) was done on the data on bubble classes for the extrudates. Significant higher percentage of bubbles ($p < 0.05$) belonging to classes 1 and 2 (i.e. small and medium size) were measured in all extrudates. In all, small and medium bubbles exceeded 95% of the total bubble area. It followed that the percentage of

bubbles belonging to class 3 (i.e. bubbles having an area greater than 3 mm²) was very low. Considering the total area (mm²), medium-size bubbles resulted in the largest area. The percentage of the total area occupied by bubbles belonging to the third class reached 18% in extrudate 3 representing the highest value among the six extrudates (Table 4.17). Although not many larger pores were measured, the high temperature extrusion might have resulted in protein denaturation and shrinkage; and these structural changes might have induced the formation of some medium pores especially around the starch granules (Pagani *et al.*, 1989).

Total porosity is defined as a measure of empty spaces in a material. It is a fraction of the volume of empty spaces over the total volume between 0 and 1 or 0 to 100%. Significant differences were observed for total porosity ($p < 0.05$) of the extrudates and this can be attributed to the differences in rice cultivar and sprouting time (Table 4.17). The porous area was significantly higher for the extruded 3 which contained the highest sprouting duration (3 day). Extrudates prepared from *Jasmine 85* rice were characterized with lower total porosity whereas *Viwonor* rice – based extrudates were generally higher. Similar results of a study conducted by Ravidran *et al.*, (2011) on rice-pea blend extrudates showed that all three gums resulted in good expanded products; increasing the inclusion of gums however ($p > 0.05$) had no effect on the degree of expansion.

Various sizes of bubbles occur during expansion of extrudates. Expansion due to extrusion is considered as a complex phenomenon which takes place at a high temperature low-moisture cooking and it results from several events including starch structural transformations and pulse transitions, nucleation, extrudate swell, bubble growth and collapse with bubble dynamics dominantly contributing to the expansion phenomenon (Fayose, 2007).

The relatively low protein concentration ranging from approximately 5 to 50 mg protein/g sample in the extrudates promoted expansion as protein concentrations in expanded extrudates are generally lower than starch concentrations to promote expansion, crispness, and increase bulk density (Day and Swanson, 2013).

The presence of relatively higher amylopectin ratio resulted in higher expansion indices in that extrudates made from *Jasmine 85* rice (Extrudates 4, 5 and 6) were characterized by higher expansion rates whereas *Viwonor* - based ones (extrudates 1, 2 and 3) generally had lower number of small and medium sized bubbles. Bubbles belonging to class 1 and 2 were dominantly present in all extrudates.

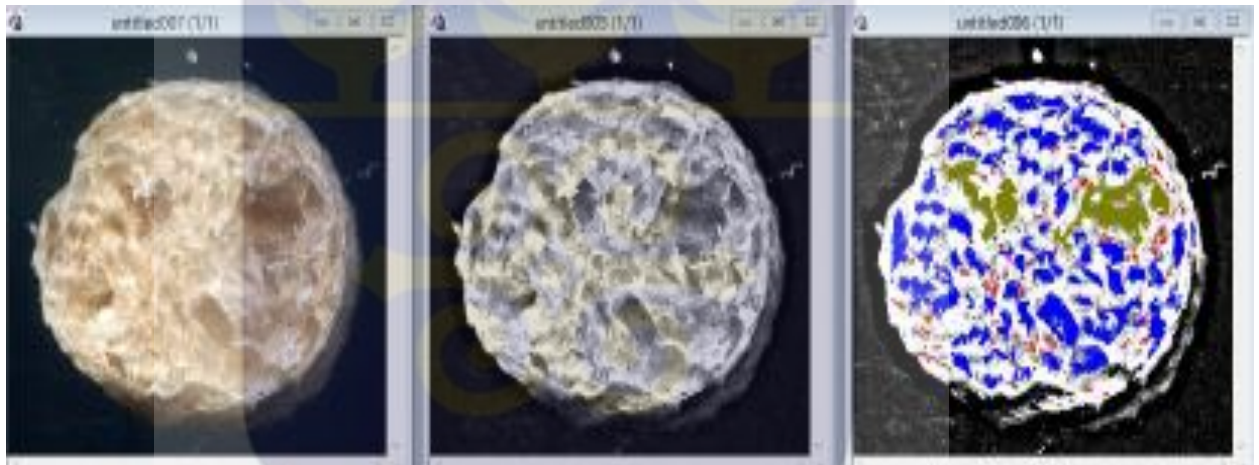


Fig 4.16: Image acquisition, standardization and image processing using the Image Pro-Plus software.

Table 4.17: Porosity of Extrudates

Extrudate	Class	Bubbles	% Bubbles	Total area (mm ²)	Total area (%)	Diameter (mm)	Circumference (mm)	Porosity (%)*
1	1	85.25	55.19	3.33	8.50	11.35 ^b	35.66 ^b	38.9 ^c
	2	67.25	44.27	33.35	84.78			
	3	0.75	0.54	2.68	6.72			
	TOTAL	153.25	100.00	39.36	100.00			
2	1	80.75	53.76	3.25	8.52	11.41 ^b	35.85 ^b	37.3 ^c
	2	67.25	45.72	31.97	83.73			
	3	0.75	0.52	2.97	7.75			
	TOTAL	148.75	100.00	38.19	100.00			
3	1	56.25	54.92	2.23	6.40	10.46 ^a	32.86 ^a	41.1 ^d
	2	43.25	43.43	26.38	75.54			
	3	1.50	1.66	6.67	18.06			
	TOTAL	101.00	100.00	35.28	100.00			
4	1	91.42	55.46	3.75	10.82	12.92 ^c	40.59 ^c	26.9 ^a
	2	71.92	44.12	28.78	81.15			
	3	0.67	0.43	2.74	8.03			
	TOTAL	164.00	100.00	35.27	100.00			
5	1	89.83	51.44	3.72	9.48	12.45 ^c	39.11 ^c	32.6 ^b
	2	82.42	48.26	34.02	85.87			
	3	0.50	0.29	1.96	4.65			
	TOTAL	172.75	100.00	39.70	100.00			
6	1	76.83	53.45	3.20	8.62	11.86 ^b	37.26 ^b	33.8 ^b
	2	64.92	46.02	31.37	84.19			
	3	0.75	0.54	2.73	7.20			
	TOTAL	142.50	100.00	37.30	100.00			

ANOVA was performed for the statistical analysis, where different superscript letters indicate significant difference

4.7 Chemical Properties of Rice - Cowpea Extrudates

4.7.1 Moisture Content (%) of Extrudates

Sprouting of cowpea significantly increased the moisture content of extrudates made from *Viwonor* rice (Extrudates 1, 2 and 3). On the hand, lower moisture contents were measured among the *Jasmine 85* - based extrudates (Extrudates 4, 5 and 6). The higher moisture content of the *Viwonor* rice significantly increased the moisture contents the extrudates made from *Viwonor* rice (Table 4.18). The addition of cowpea flour to both rice samples (*Viwonor* and *Jasmine 85*) resulted in reduced the moisture content notably as the germination time increased (Table 4.18).

Table 4.18: Moisture Content (%) of Extrudates

Sample	Rice Variety	Cowpea Treatment	Moisture Content (%)
Extrudate 1	<i>Viwonor</i>	Unsprouted (0 day)	7.48± 0.03 ^b
Extrudate 2	<i>Viwonor</i>	2 day Sprouted	8.08 ±0.18 ^c
Extrudate 3	<i>Viwonor</i>	3 day sprouted	9.17 ±0.67 ^d
Extrudate 4	<i>Jasmine 85</i>	Unsprouted(0 day)	7.40 ±0.02 ^b
Extrudate 5	<i>Jasmine 85</i>	2 day Sprouted	7.28 ±0.14 ^b
Extrudate 6	<i>Jasmine 85</i>	3 day sprouted	6.44 ±0.17 ^a

4.7.2 Protein Content of Rice - Cowpea Extrudates

Protein quantities of the rice - sprouted cowpea extrudates are described in Table 4.19. Rice variety significantly affected ($p < 0.05$) protein contents of the extrudates. The relatively lower protein content of *Viwonor* rice reflected in the decreased protein contents in the *Viwonor* rice-based extrudates. Whereas the *Jasmine 85* rice gave higher protein content among extrudates made from *Jasmine 85* rice. Increasing sprouting duration of cowpea seeds resulted in decrease in protein content of all extrudates irrespective of the

buffer. This suggests that the storage proteins in the cowpea (mostly globulins) have been metabolized into the growth of the plant during sprouting (Harris and Chrispeels, 1975; Pflanzengenetik and Kulturpflanzenforschung, (2001).

Table 4.19: Protein Content of Rice - Cowpea Extrudates

Sample	Cowpea sprouting time (days)	Rice Variety	Buffer	Protein content mg/g sample
Extrudate 1	0	<i>Viwonor</i>	NaCl	3.53 ±0.05 ^a
Extrudate 1	0	<i>Viwonor</i>	Urea	19.67 ±0.26 ^b
Extrudate 1	0	<i>Viwonor</i>	DTT	36.36 ±0.23 ^c
Extrudate 2	2	<i>Viwonor</i>	NaCl	2.81 ±0.03 ^a
Extrudate 2	2	<i>Viwonor</i>	Urea	18.04 ±0.82 ^b
Extrudate 2	2	<i>Viwonor</i>	DTT	34.46 ±0.15 ^c
Extrudate 3	3	<i>Viwonor</i>	NaCl	2.46 ±0.10 ^a
Extrudate 3	3	<i>Viwonor</i>	Urea	21.70 ±0.05 ^b
Extrudate 3	3	<i>Viwonor</i>	DTT	39.67 ±0.46 ^c
Extrudate 4	0	<i>Jasmine 85</i>	NaCl	5.87 ±0.00 ^a
Extrudate 4	0	<i>Jasmine 85</i>	Urea	30.94 ±0.20 ^b
Extrudate 4	0	<i>Jasmine 85</i>	DTT	48.48 ±0.00 ^c
Extrudate 5	2	<i>Jasmine 85</i>	NaCl	1.26 ±0.19 ^a
Extrudate 5	2	<i>Jasmine 85</i>	Urea	14.06 ±0.51 ^b
Extrudate 5	2	<i>Jasmine 85</i>	DTT	34.35 ±2.00 ^c
Extrudate 6	3	<i>Jasmine 85</i>	NaCl	1.46 ±0.01 ^a
Extrudate 6	3	<i>Jasmine 85</i>	Urea	13.70 ±0.10 ^b
Extrudate 6	3	<i>Jasmine 85</i>	DTT	32.07 ±0.15 ^c

4.7.3 Protein Solubility of Rice - Cowpea Extrudates

Table 4.9 shows that the amount of proteins solubilized in saline buffer was low for all samples (extrudates) and it increased after addition of urea and urea/DTT to the extraction medium. In all the extrudates, disulphide bonds played an important role in stabilizing the

protein network, as indicated by the significant increase of the solubilized proteins after addition of the reducing agent (DTT) to the extraction medium.

Sprouted cowpea flour significantly influenced the overall protein organization with respect to rice variety ($p < 0.05$). No differences could be identified in the overall organization of protein aggregates stabilized by hydrophobic interactions and disulphide bonds, regardless of the source and nature of the cowpea (sprouted or non-sprouted) with respect to the *Viwonor* rice - based extrudates. On the contrary, significantly higher amount of proteins was extracted from samples prepared with *Jasmine* - 85 rice and non-sprouted cowpea (Extrudate 4), suggesting that the sprouted cowpea proteins impaired the formation of a compact protein network (with respect to *Jasmine*-85 rice). The addition of sprouted cowpea flours to *Jasmine* - 85 based extrudates resulted in the formation of a more compact protein network that appeared more similar to that of the whole rice in figure 4.1.

Protein solubility in extrudates was generally low in saline buffer (Fig 4.17). Extrudates made with *Viwonor* rice and unsprouted cowpea had much lower protein solubility than those made with *Jasmine* 85 rice. Germination of cowpea decreased protein solubility of extrudates produced from *Jasmine* 85 rice while it increased the solubility of *Viwonor* rice – based extrudates. The solubility of the extrudates generally improved when urea buffer was used. The solubility further increased when the Dithiothreitol was combined with the urea buffer (Fig. 4.17).

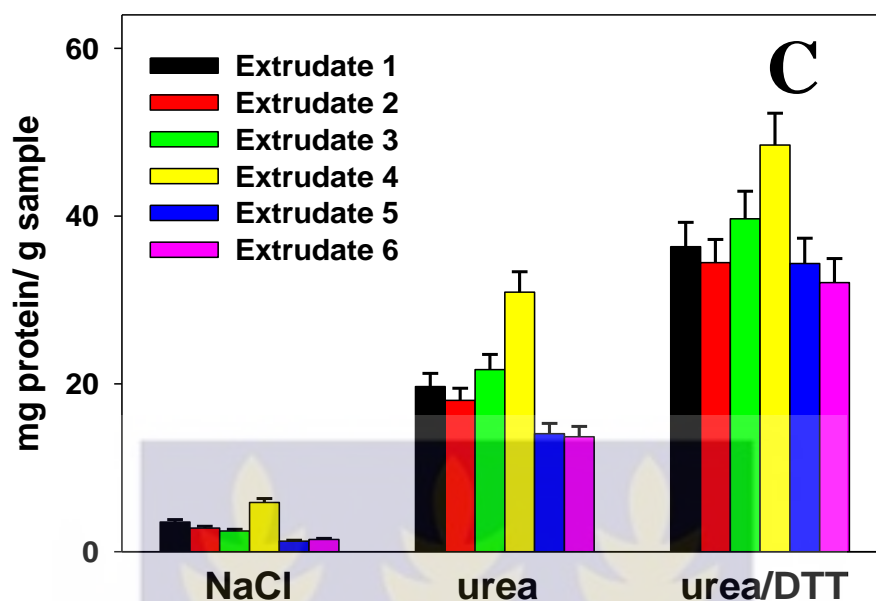


Fig 4.17: Soluble proteins in saline buffer (NaCl), containing urea and urea/DTT, when indicated from extrudates (panel C).

4.7.4 SDS-PAGE of Protein Extracts from Rice - Cowpea Extrudates

Figure 4.18 shows the protein bands of *Viwonor* rice - based extrudates. The figure shows that proteins from extrudates of unsprouted cowpea (Extrudate 1) were extracted by the saline buffer while proteins of sprouted cowpea- extrudates (Extrudate 2 and 3) could not be extracted. The saline buffer (A) showed protein bands of 30KDa MWT in the unsprouted cowpea – based extrudate (Extrudate 1) which however disappeared upon sprouting as shown in the protein bands in buffer A for Extrudate 2 and 3. Cowpeas consist largely of albumin and globular proteins (Fotso *et al.*, 1994). Globular proteins are soluble in neutral salt solutions (almost insoluble in water) while albumins are soluble in neutral salt free water (de Man, 1999). The observed extracted proteins by the saline buffer from the extruded cowpea and rice (Extrudate 1) could largely be globulins from both cowpea and rice. The saline buffer (A) however could not extract proteins from extrudates of sprouted cowpea. It is possible that during sprouting the 30 KDa were metabolized.

Figure 4.18 and Figure 4.19 show proteins deriving from rice and cowpea in the tracings of the extrudates. These results show that both rice and cowpea proteins contributed to extrudates protein structure. No new protein bands that was neither in the rice nor could cowpea bands be identified in the extrudates suggesting that extrusion conditions were not severe enough to create the formation of new proteins.

Rice – Cowpea Extrudates

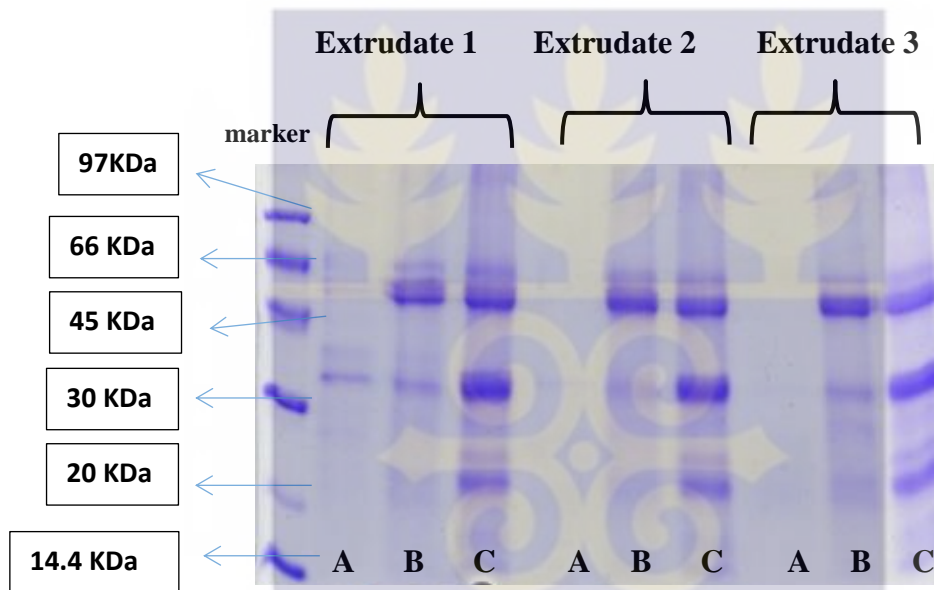


Fig 4.18: SDS-PAGE of proteins extracted from ground extrudates. Proteins were solubilized in: saline buffer (A); buffer in the presence of 6 M urea (B); buffer in the presence of 6 M urea and 10 mM DTT (C). Samples were treated in the presence of β -mercaptoethanol prior to protein separation

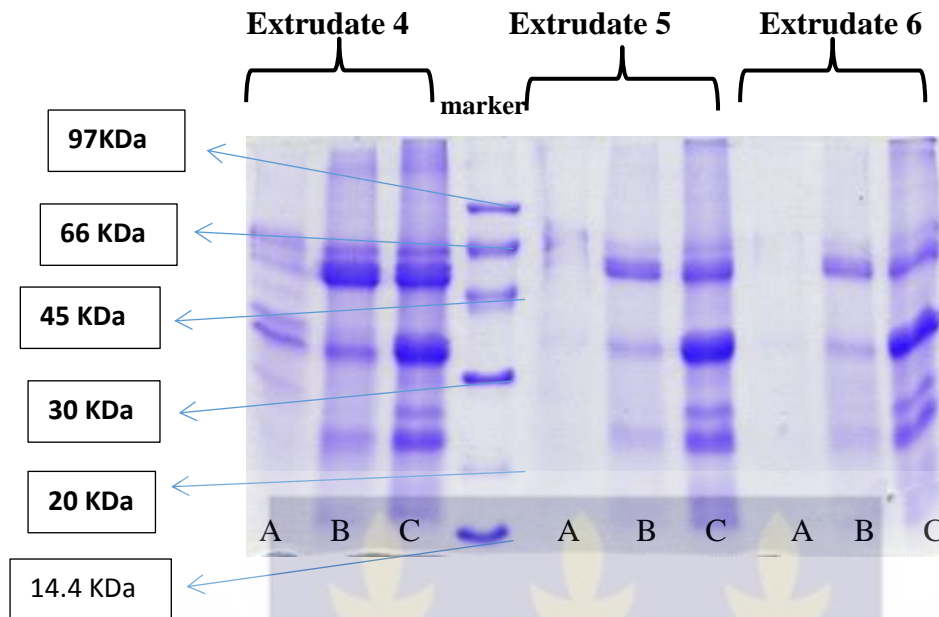


Fig 4.19: SDS-PAGE of proteins extracted from ground extrudates. Proteins were solubilized in: saline buffer (A); buffer in the presence of 6 M urea (B); buffer in the presence of 6 M urea and 10 mM DTT (C). Samples were treated in the presence of β -mercaptoethanol prior to protein separation

4.7.5 In vitro Digestibility of Proteins (pepsin and pancreatin) of Rice - Cowpea Extrudates

Figure 4.20 shows that extrudates made using *Viwonor* rice had a faster digestion rate by the pepsin and pancreatin enzymes than extrudates by the *Jasmine 85*. Sprouting of the cowpea also had an influence on the digestibility of the extrudates. Extrudates from unsprouted cowpea showed the highest rate of hydrolyses by pepsin and the least rate of hydrolyses by pancreatin was observed for extrudates 3. This can be related to the proteolytic activities during germination which generated peptides that resulted in more pepsin-sensitive proteins than the whole proteins.

A similar behavior of protein susceptibility to pepsin and pancreatin was observed in the case of the extrudates prepared with high-quality *Jasmine 85* rice (Fig. 4.20); whereas peculiar curves described the release of peptides from samples prepared with low-quality *Viwonor* rice. This observation highlighted the significant effect of rice protein quality on

the structure of the extrudates, regardless of the cowpea source (germinated or not). Extrudates made from *Jasmine 85* rice were generally characterized with lower pancreatic digestibility. Extrusion cooking significantly increased protein digestibility (among the extrudates) as this has been reported by Day and Swanson, (2013) that in vitro protein digestibility of plant protein-based foods are generally enhanced by extrusion.

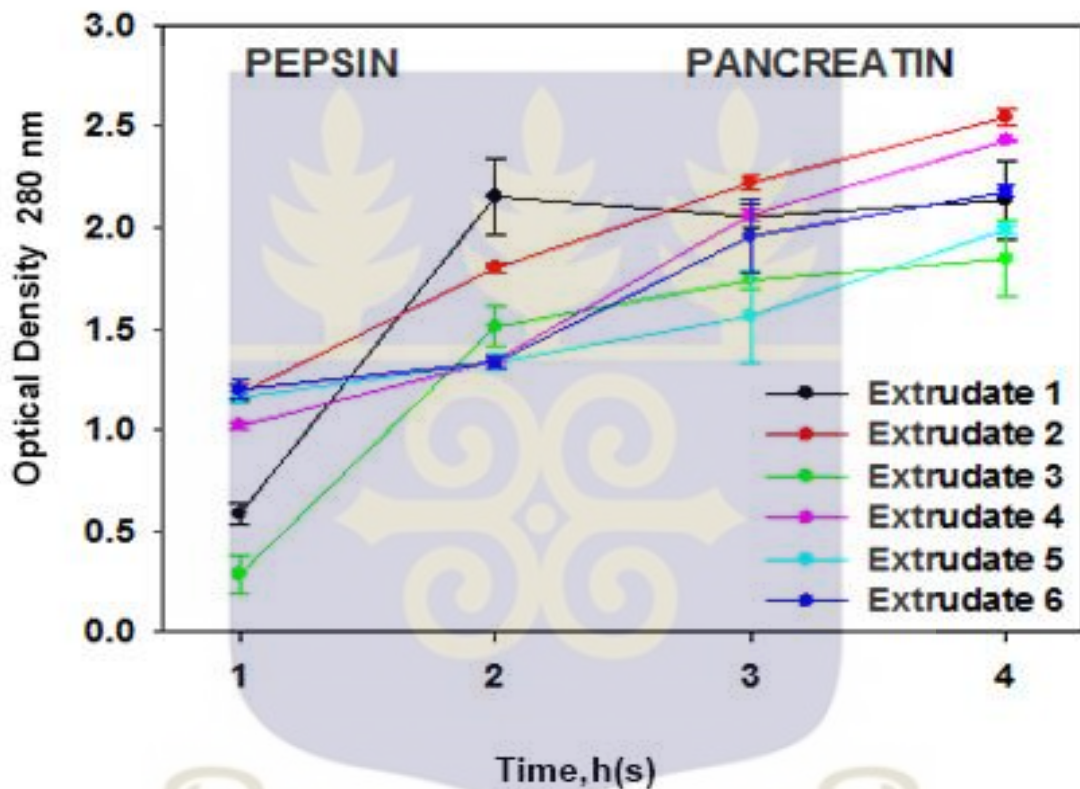


Fig 4.20: In vitro Pepsin and Pancreatin Digestion of Extruded Samples.

4.7.6 Accessible Thiols of Rice and Cowpea Extrudates

Increase in germination time resulted in a looser structure among extrudates made from *Vivonor* rice (low-quality rice) whereas a more compact structure could be underlined for the samples produced with *Jasmine 85* variety (Fig. 4.21). This observation tells the important role of high q

quality protein - deriving from *Jasmine 85* rice – in the definition of extrudates structure. The ANOVA results showed significant difference in the accessible thiols of the extrudates.

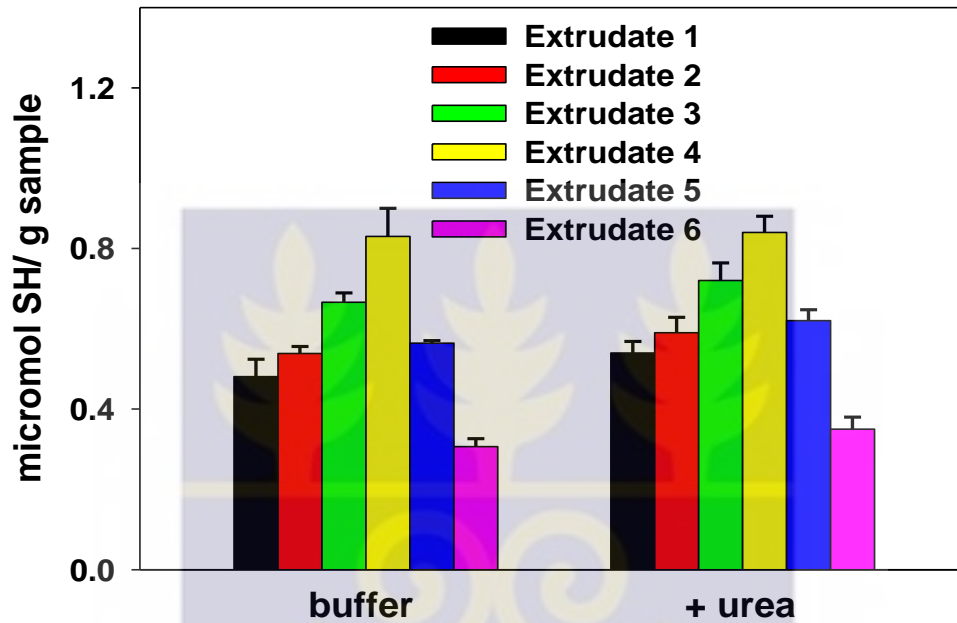


Fig 4.21: Accessible Thiols of Extrudates

4.8 Physico-functional Properties of Rice - Cowpea Extrudates

Physico-functional property of food is that property that characterizes the structure, quality, nutritional value and acceptability of the food product. Information on physico-functional property of foods plays an important role in the production of diversified food products.

4.8.1 Water Absorption Index (WAI) of Rice - Cowpea Extrudates

The measurement of water absorption index (WAI) gives an indication of the amount of water absorbed by a sample. It is estimated as the weight difference between the samples exposed to water and the dried samples (Espert *et al.*, 2004). Figure 4.22 show the water absorption index of the extrudates. There was no significant difference ($P > 0.05$) in WAI

index of the extrudates indicating that sprouting time and rice cultivar did not influence the water absorption pattern of the extrudates. Water absorption depends on the availability of hydrophilic groups which binds to the water molecule (Gomez and Aguilera, 1983). This suggests that the available hydrophilic groups within the starch molecules of the extrudates were similar.

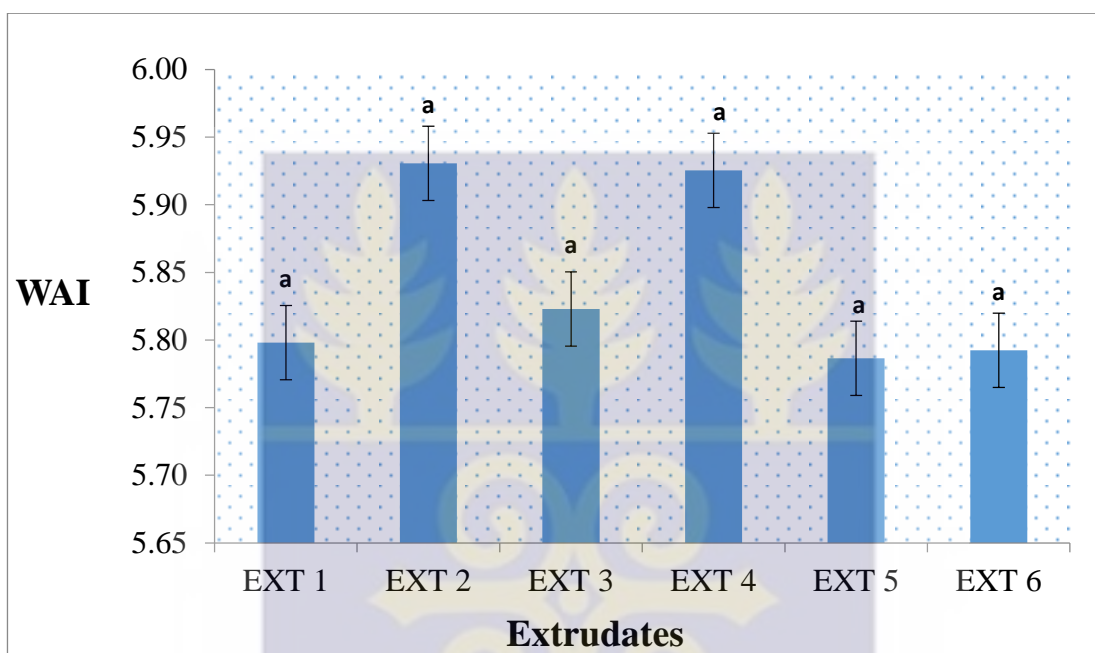


Fig 4.22: Water Absorption Index of Rice-Cowpea Extrudates

4.8.2 Water Solubility Index (WSI) of Rice-Cowpea Extrudates

Water solubility index (WSI) is defined by the amount of soluble solids remaining in the liquid phase when starch is submerged in water (Diop *et al.*, 2011). It measures the degree of starch conversion during extrusion which is the amount of soluble polysaccharide released from the starch component after extrusion (Ding *et al.*, 2005). Rice cultivar and sprouting time significantly affected ($P < 0.05$) the WSI of the extrudates. Relatively lower WS index was measured among the extrudates made from *Viwonor* rice whereas the *Jasmine 85* based extrudates were higher. Amylopectin has been reported by Wang and Wang (2003) to contribute mostly to swelling and pasting property of starch granules

while amylose retards swelling. This confirms the measurement of lower WS index among of the extrudates made from amylose-rich rice flour (*Viwonor* rice). *Jasmine 85* rice with higher amylopectin content as expected gave higher WS index.

Hagenimana *et al.*, (2006) reported that low moisture content during extrusion cooking is associated with increased amount of degraded starch granules resulting in the formation of increased water-soluble products; and this phenomenon is caused by shear fragmentation of the starch during extrusion cooking. This situation was observed to have taken place as higher WS values were measured among extrudates produced from *Jasmine-85* rice (extrudates 4, 5 and 6) with lower moisture contents.

Increasing sprouting time significantly decreased ($p < 0.05$) the WSI of the *Viwonor* - based extrudates. Extrudates produced from the highest germination time of cowpea measured the least WS index irrespective of rice cultivar. It is possible that at the highest germination time, the degraded starches and proteins in the cowpea flour samples were insoluble.

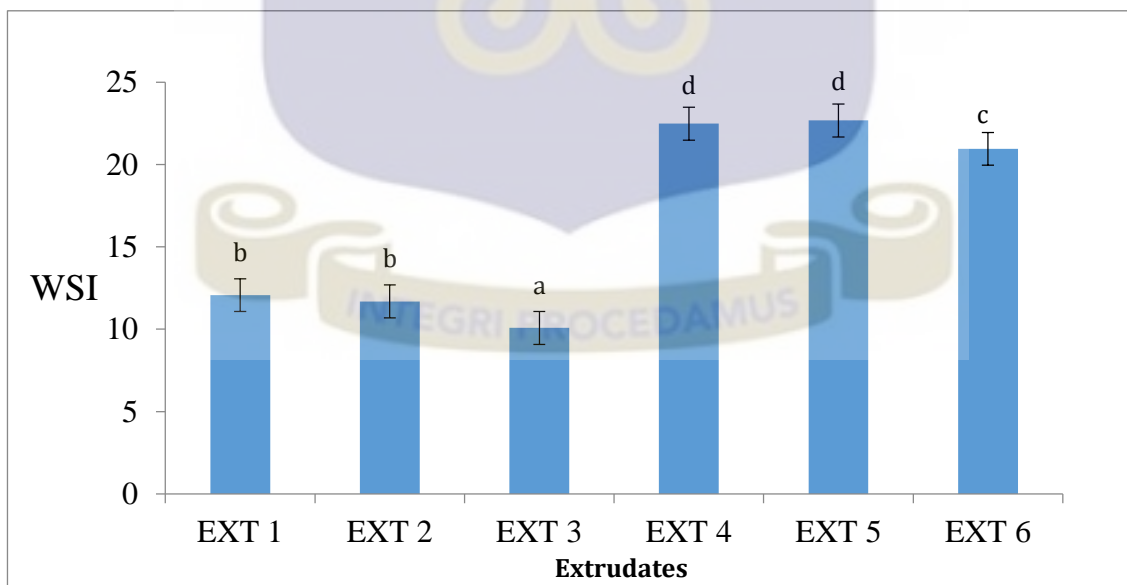


Fig 4.23: Water Solubility Index (WSI) of Rice – Cowpea Extrudates

4.9 Instrumentation Sensory analysis

4.9.1 Electronic Nose Analysis

The perception of volatile compounds by the human nose is of great importance in evaluating the quality of foods. The Electronic Nose (EN) is an instrument that mimics the human olfactory perception and provide an odour print of the samples. It is equipped with an array of non-selective and broad spectrum chemical sensors used for the headspace of liquid or solid sample analysis (Laureati *et al.*, 2010). Electronic nose systems comprise a sophisticated hardware, with sensors, electronics, pumps, air conditioner, flow controller, etc. in addition to software for hardware monitoring, data pre-processing and statistical analysis (Schaller *et al.*, 1998). The instruments operating system of the electronic nose is on a similar principle as the human nose. The system ensures complete conventional analyses of volatile compounds by sensory methods and by traditional analytical techniques (Schaller *et al.*, 1998). The statistical techniques used are based on commercial or specially designed software using pattern recognition routines like Principal Component Analysis (PCA).

In this study the sensor array of the device were composed of 10 Metal Oxide Semiconductor (MOS) type chemical sensors. They include; W1C (aromatic) W5S (broadrange) W3C (aromatic) W6S (hydrogen) W5C (arom-aliph) W1S (broad-methane) W1W (sulphur-organic) W2S (broad-alcohol) W2W (sulph-chlor) W3S (methane-aliph). The sensor response is expressed as resistivity (Ω). The statistical techniques used are based on commercial or specially designed software using pattern recognition routines like Principal Component Analysis (PCA). Principal Components Analysis (PCA) is a procedure that permits to extract useful information from the data, to explore the data structure, the relationship between objects, the relationship between objects and variables and the global correlation of the variables.

The electronic nose was used to evaluate the aromatic profile of the extrudates 1-6. For each sample, the electronic nose sensor responses were collected, elaborated by PCA and performed in a correlation matrix to achieve a partial visualization of the data set in a reduced dimension. Figure 4.24 shows the PCA score plot of the 1-6 extruded samples distribution in the area defined by the first two principal components (PCs) that explain the 80.1% of total variance. The score plot shows that samples were dispersed along the first principal components and there is a clear separation of extrudates 1 and 4 (containing unsprouted cowpea) from 2, 3, 5, and 6 (containing sprouted cowpea). The second principal component (PC2) of the score plot was able to distinguish the extrudates - based on rice variety (thus *Viwonor* and *Jasmine 85*).

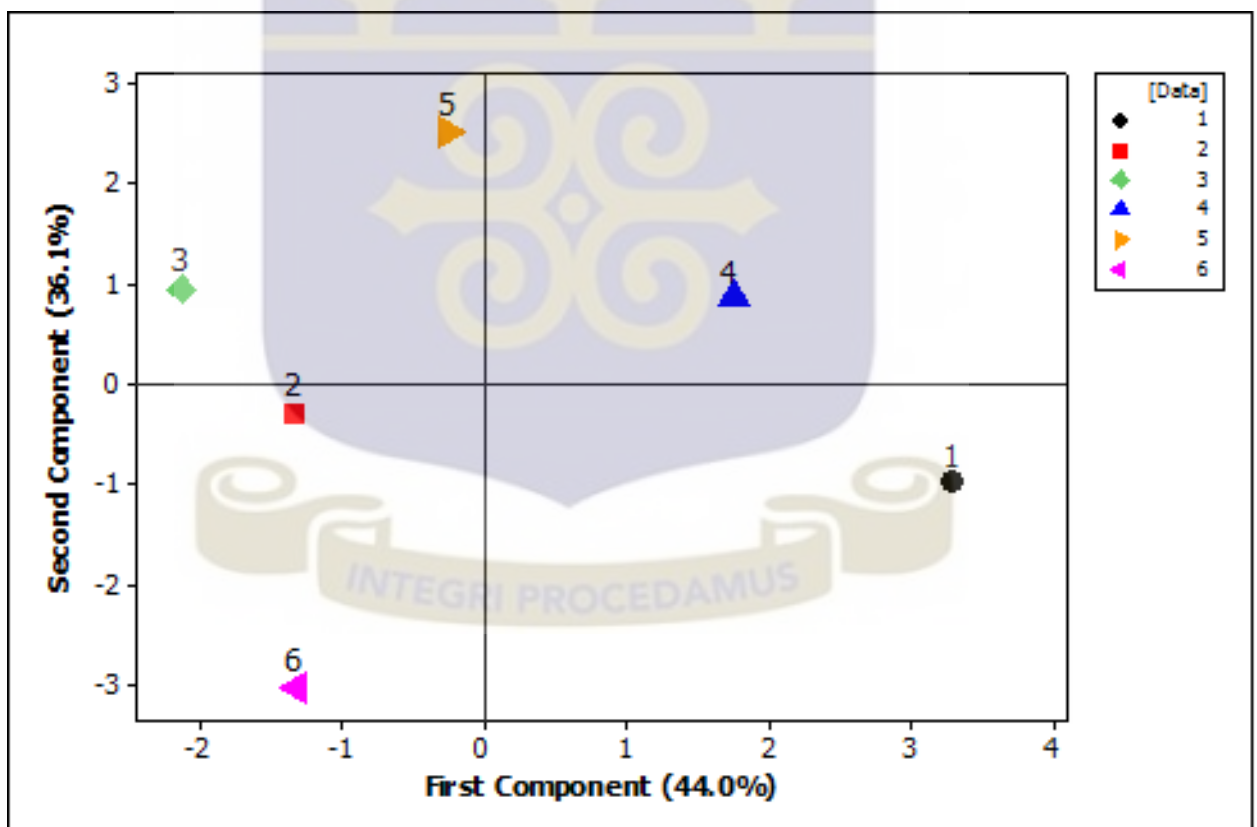
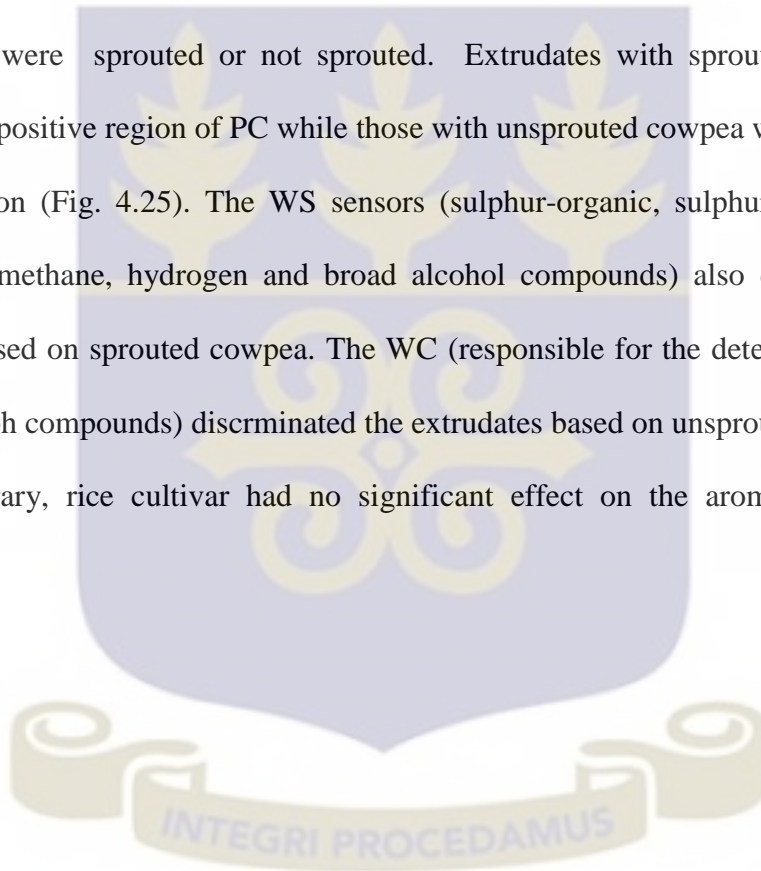


Fig 4.24: PCA Score Plot of Extrudates

The WC and WS sensors contributed to the discrimination of aroma profile of extrudates 1 and 4 from 2, 3 5 and 6 respectively (Fig 4.25). Discrimination of aroma profile of extrudates were based on sprouted cowpea and unsprouted cowpea as components in the extrudates. The first principal component (PC1) discriminated the extrudates based on sprouted cowpea and unsprouted cowpea.

Results showed that the aroma profile of the extrudates was significantly affected ($p < 0.05$) by cowpea sprouting time. The discriminations of the extrudates were based on whether the cowpeas were sprouted or not sprouted. Extrudates with sprouted cowpea were loaded in the positive region of PC while those with unsprouted cowpea were loaded in the negative region (Fig. 4.25). The WS sensors (sulphur-organic, sulphur-chlor, methane-aliph, broad methane, hydrogen and broad alcohol compounds) also discriminated the extrudates based on sprouted cowpea. The WC (responsible for the detection of aromatic and arom-aliph compounds) discriminated the extrudates based on unsprouted cowpea. On the contrary, rice cultivar had no significant effect on the aroma profile of the extrudates.



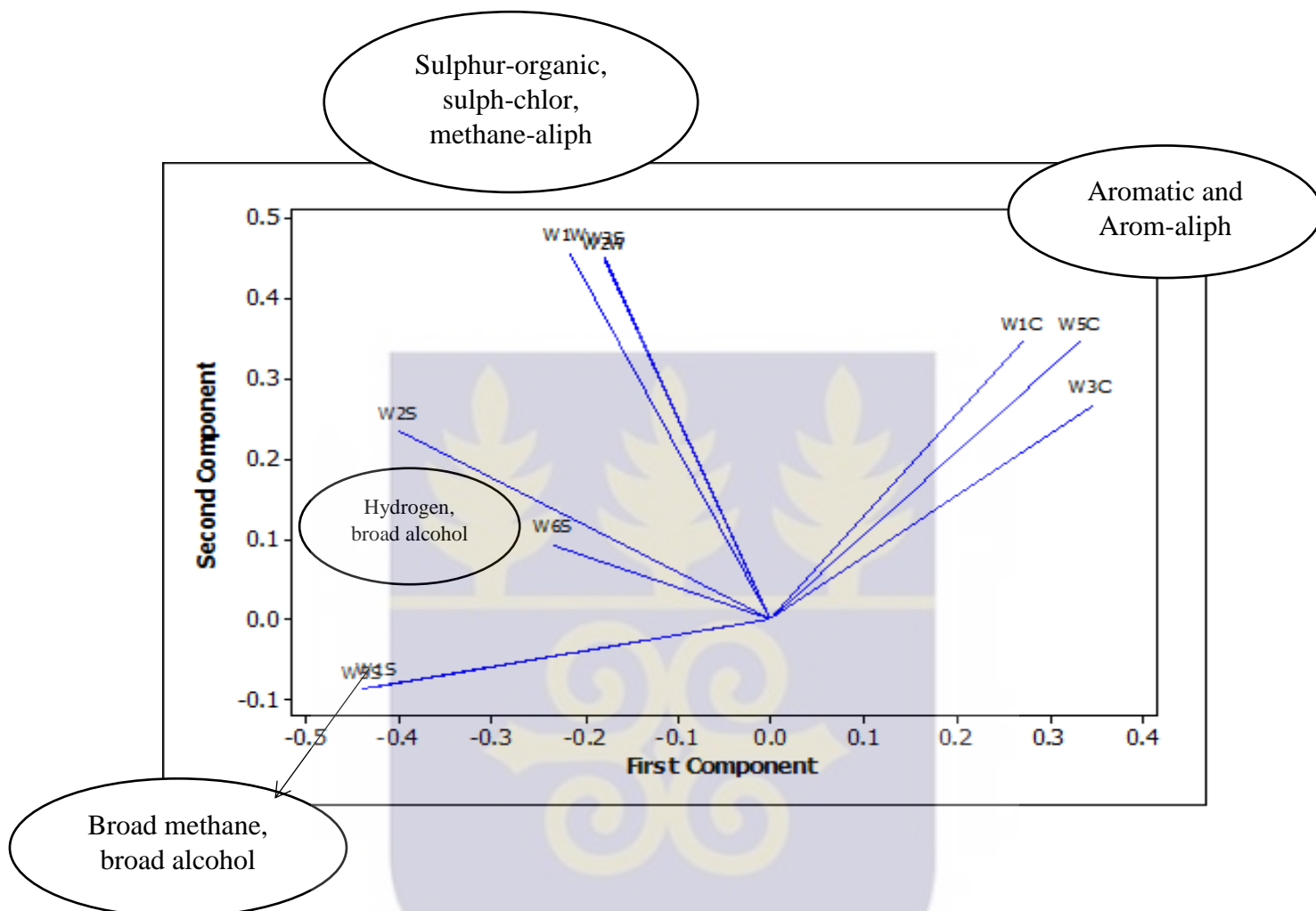


Fig 4.25: PCA Loading Plot of Extrudates (Electronic Nose)

4.9.2 Electronic Tongue Analysis

The Electronic Tongue (ET) is a liquid device that mimics the taste sensing mechanism and information processing of gustatory system (Laureati *et al.*, 2010). It comprises an array of sensors that are specific for liquid and are able to classify four basic qualities (sourness, saltiness, bitterness and umami) taste (Toko, 2000). The technique employs an array of chemical sensors and pattern recognition system for the classification of liquid samples. It is widely applied in the food, medicine and chemical industries. Other applications of the Electronic Tongue are in environmental monitoring and quality control assessment.

The taste values collected by electronic tongue (ET) were elaborated by Principal Component Analysis (PCA). PCA was used for explorative data analysis in order to achieve a partial visualization of the data set in a reduced dimension. PCA was performed in correlation (the variables were scaled). Two figures were obtained from the elaboration; PCA-Score plot representing the relationship among the samples, and PCA-loading plot showing the relationship among the variables and how they influence the system.

Figure 4.26 shows the score plot of the extrudates defined by the first two Principal Component of total variance of 89.7%. There is a clear distinguishing of extrudate by the first (PC1) and second (PC2) Principal Components. In particular, extrudates 2 and 3 located in the negative part of the PC1 are characterized by sourness and are perceived less astringent and bitter. However, these extrudates were less characterized by umami and saltiness tastes (Fig 4.27). This shows that increasing sprouting time of cowpea significantly influenced the taste of the *Viwonor*-based extrudates.

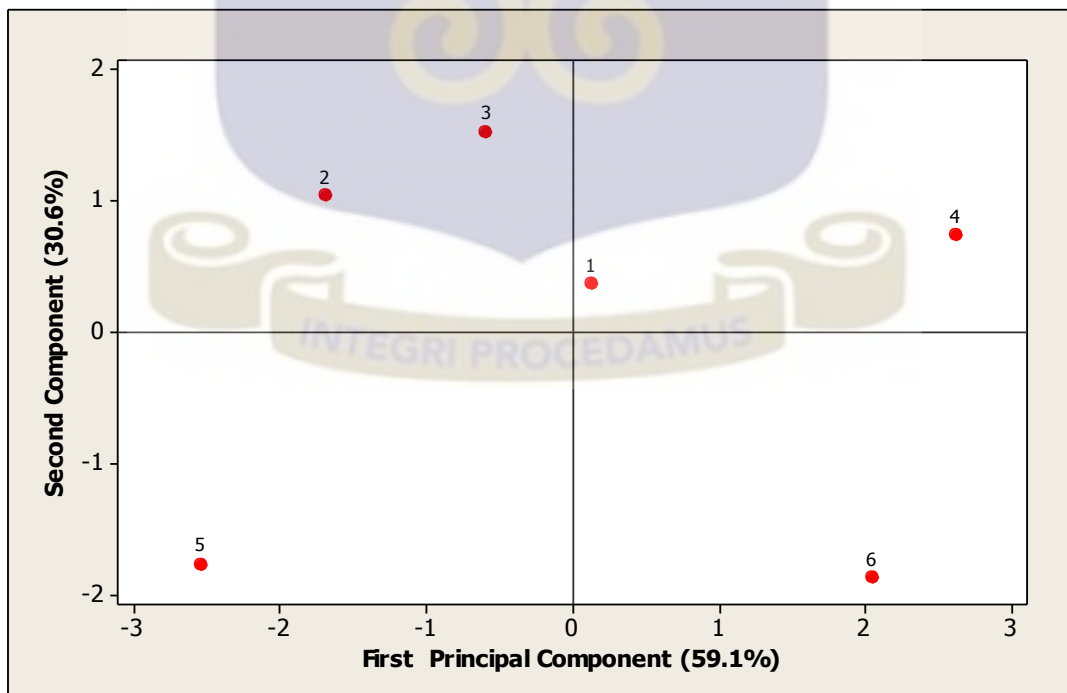


Fig 4.26: PCA Score Plot of Extrudates (Electronic Tongue)

Sprouting of the legume significantly influenced ($p < 0.05$) the taste of the extrudates irrespective of the rice cultivar. In the positive part of the PC1 extrudates 1 and 4 were perceived to be more bitter and astringent tastes and were characterized by umami and saltiness. The addition of sprouted cowpea flour to both rice samples produced extrudates with less bitter and astringent tastes. The measurement of less bitter and astringent tastes could be due to the enzymatic breakdown of complex molecules of proteins, carbohydrates and fats into simple sugars, peptides and amino acids that took place at the germination stage of the cowpea (Obatolu *et al.*, 2000; Kuo *et al.*, 2004; Moongngarm and Saetung, 2010).

Increasing sprouting time minimized the level of sourness and bitterness in both *Viwonor* and *Jasmine* - based extrudates. This was more evident in extrudates 1 and 4 which exhibited much more bitter and stringent tastes characterized by umami and saltiness as this may be due to the presence of relatively higher levels of macromolecules and anti-nutritional factors in the unsprouted cowpea (Uwaegbute *et al.*, 2000).

Discrimination of extrudates by PC2 was based on rice variety. Extrudates 5 and 6, positioned in the negative part of PC2 were discriminated by the astringency and bitterness aftertastes. Intermediate taste of bitterness and astringency were measured among the *Jasmine 85* - based extrudates as sprouting time increased. The *Viwonor* rice – based extrudates were discriminated by PC 2 in the positive region of the score plot (Fig. 4. 26) and were characterized by sourness, saltiness, astringency, umami and bitterness aftertaste (Fig 4.27).

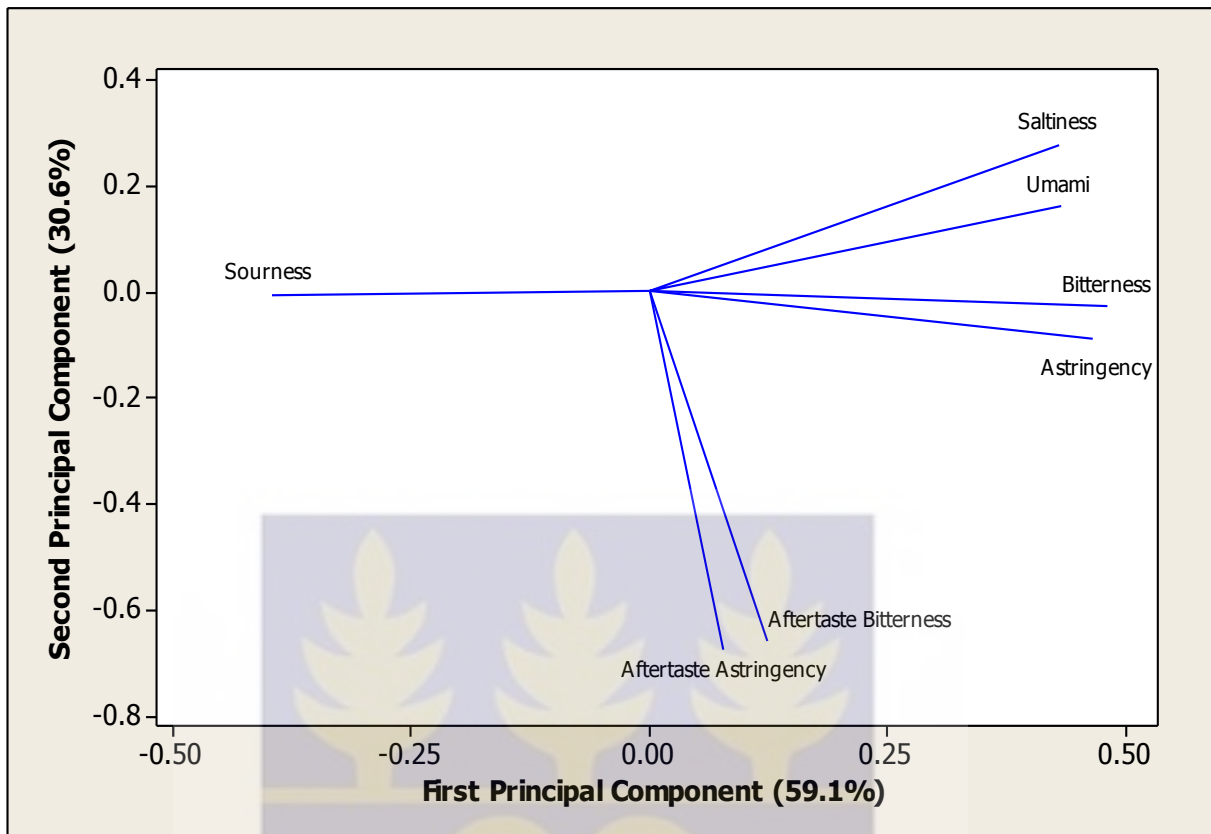


Fig 4.27: PCA Loading Plot of Extrudates (Electronic Tongue)

In summary, extrudates made with unsprouted cowpea flours (extrudates 1 and 4) were perceived to be less bitter and astringent aftertaste and were characterized by umami and saltiness. *Viwonor* - based extrudates enriched with sprouted cowpea (extrudates 2 and 3) were characterized by sourness whereas *Jasmine 85* - based extrudates with sprouted cowpea (extrudates 5 and 6) were characterized by astringency and bitter aftertaste.

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Rice cultivars significantly influenced ($p < 0.05$) its physical properties. Lower % head rice was measured in *Viwonor* rice. There was no significant difference in grain size and shape of both rice cultivars. Relatively higher apparent amylose and Gelatinization Temperature (GT) class was measured in *Viwonor* rice. Rice variety significantly affected ($p < 0.05$) the quantity of proteins extracted in all 3 (saline, Urea and DTT) buffers. Lower protein content was extracted in *Viwonor* rice. *Jasmine 85* rice proteins were generally more soluble than *Viwonor* rice proteins in all three buffers. The SDS-PAGE tracings of extracts from rice samples showed no differences in the composition of the proteins. Protein bands were similar for both *Viwonor* and *Jasmine 85* rice varieties. The protein hydrolyses trends show that *Viwonor* rice tendered to have lower digestibility than *Jasmine 85* rice to pepsin but in particular the pancreatin hydrolyses. The accessible thiols measured in both *Viwonor* and *Jasmine 85* rice showed that the overall protein organizations were similar.

Protein quantities and solubilities of cowpea significantly decreased ($p < 0.05$) with increasing sprouting time across all buffers. The solubility of cowpea proteins in saline was generally low but improved upon the addition of urea and the reducing agent dithiothreitol (DTT). There was no significant variability in the number of protein bands for each germination day and by the different buffer solutions. Generally, cowpea proteins of higher molecular weight (45 and 66 KDa) were mostly extracted in all buffers. Cowpeas that were sprouted for three days were far more digestible by pepsin and pancreatin enzymes than those that were not sprouted for that long. Increasing germination

time of cowpea seeds significantly decreased ($p < 0.05$) the amount of accessible thiols with or without the denaturant.

13 flour formulations were obtained from rice and sprouted cowpea using the Central Composite Rotatable Design (CCRD) and extruded. Consumer preference test was done on the extrudates. ANOVA was performed and the mean acceptability score was used to select the 3 most preferred extrudates. Extrudate 7 (22% 2 day sprouted cowpea + 78 *Viwonor* rice) was rated the most preferred extrudate. This was followed by extrudate 1 and 2 with 30% unsprouted cowpea + 70% *Viwonor* rice and 30% 3 day sprouted cowpea + 70 *Viwonor* rice. The corresponding flour formulation for the best 3 extrudates was used in extruding additional 3 extrudates from *Jasmine 85* rice (control).

Germination of cowpea decreased protein content and solubility of extrudates produced from *Jasmine 85* rice while it increased the solubility of *Viwonor* rice – based extrudates. The SDS-PAGE results show that both rice and cowpea proteins contributed to the protein structure of the extrudates. No new protein bands that was neither in the rice nor could cowpea bands be identified in the extrudates. Extrudates produced from unsprouted cowpea (Extrudates 1 and 4) showed the highest rate of hydrolyses by pepsin while the sprouted cowpea – based extrudates were low. Extrudates made from *Jasmine 85* rice were generally characterized with lower pancreatin digestibility. Sprouting of cowpea seeds significantly increased the accessible thiols in the *Viwonor* rice - based extrudates while it decreased extrudates produced from the *Jasmine 85* rice.

Extrudates made from *Jasmine 85* rice were characterized with higher expansion ratios and bulk densities. *Viwonor* - based extrudates (extrudates 1, 2 and 3) measured higher redness (a^*) and yellowness (b^*) indices with lower lightness (L^*) compared with *Jasmine 85*-based ones. Lower hardness values were measured among the *Jasmine 85* - based extrudates. Small and medium sized bubbles belonging to class 1 ($0.01 - 0.1 \text{ mm}^2$) and 2

(0.1 – 3 mm²) were dominantly present in all extrudates. There was no significant difference ($P>0.05$) in WAI index of the extrudates. Rice cultivar and sprouting time significantly affected ($P <0.05$) the WSI of the extrudates.

The aroma profile of the extrudates (based on the Electronic Nose) was significantly affected ($p<0.05$) by cowpea sprouting time. The WS sensors (sulphur-organic, sulphur-chlor, methane-aliph and hydrogen) discriminated the extrudates based on sprouted cowpea whereas the WC (responsible for the detection of aromatic and arom-aliph compounds) discriminated the extrudates based on unsprouted cowpea. From the Electronic Tongue analysis, extrudates made with unsprouted cowpea flours (extrudates 1 and 4) were perceived to be less bitter and astringent aftertaste. *Viwonor* - based extrudates enriched with sprouted cowpea (Extrudates 2 and 3) were characterized by sourness whereas *Jasmine 85* - based extrudates with sprouted cowpea (extrudates 5 and 6) were characterized by astringency and bitter aftertaste.

5.2 Recommendations

- Further studies should be carried out to find the effect of extrusion cooking on the levels of nutrients and the physicochemical properties of the rice – sprouted cowpea extrudates.
- Shelf-life studies should be carried out on the extrudates to ascertain their keeping quality

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APPENDICES

Appendix 1: Results of Milling Recovery (% head rice)

Rice Variety	Treatment	Replicate	% Head Rice	Av. % Head Rice	Av. % Broken Rice
<i>Viwonor</i>	Unpolished	1	46.30	48.07	53.70
<i>Viwonor</i>	Unpolished	2	49.85		
<i>Jasmine 85</i>	Polished	1	62.37	62.28	57.63
<i>Jasmine 85</i>	Polished	2	62.18		

Appendix 2: ANOVA Table for % Head Rice

Rive Variety	N	Mean	St Dev	SE Mean	P-Value
Jasmine 85	5	62.290	0.292	0.13	0.000
Viwonor	5	47.80	1.37	0.61	

Appendix 3: Grain Dimension and Chalkiness

Rice Variety	Treatment	Replicate	L mm	W mm	L/W	% Chalky Area
<i>Viwonor</i>	Unpolished	1	6.570	2.230	2.946	7.46
<i>Viwonor</i>	Unpolished	2	6.502	2.032	3.20	7.21
<i>Jasmine 85</i>	Polished	1	6.527	2.245	2.907	11.74
<i>Jasmine 85</i>	Polished	2	6.585	6.585	2.915	11.81

Appendix 4: ANOVA Table for Grain Length/Width

Rive Variety	N	Mean	St Dev	SE Mean	P-Value
Jasmine 85	5	2.9346	0.0283	0.013	0.164
Viwonor	5	3.033	0.141	0.063	

Appendix 5: Results of Grain Hardness

Rice Variety	Treatment	Replicate	Hardness	Av. Hardness	Std. Dev
<i>Viwonor</i>	Unpolished	1	8.85	8.65	0.28
<i>Viwonor</i>	Unpolished	2	8.45		
<i>Jasmine 85</i>	Polished	1	6.45	6.40	0.08
<i>Jasmine 85</i>	Polished	2	6.34		

Appendix 6: ANOVA Table for Grain Hardness

Rive Variety	N	Mean	St Dev	SE Mean	P-Value
Jasmine 85	5	6.450	0.200	0.089	0.000
Viwonor	5	8.622	0.296	0.13	

Appendix 7: Results of Alkaline Spreading Value (ASV) of Rice Samples

Rice Variety	Treatment	Replicate	ASV	Av. ASV	Gelatinization Temperature Class
<i>Viwonor</i>	Unpolished	1	5.50	5.34 ±0.23	Inter mediate
<i>Viwonor</i>	Unpolished	2	5.17		
<i>Jasmine 85</i>	Polished	1	7.00	6.92 ±0.12	Low
<i>Jasmine 85</i>	Polished	2	6.83		

Appendix 8: Results of Apparent Amylose of Rice Samples

Rice Variety	Treatment	Replicate	Apparent Amylose	Av. Amylose	Gelatinization Temperature Class
<i>Viwonor</i>	Unpolished	1	18.86	18.82	Intermediate
<i>Viwonor</i>	Unpolished	2	18.77		
<i>Jasmine 85</i>	Polished	1	15.11	15.14	Low
<i>Jasmine 85</i>	Polished	2	15.16		

Appendix 9: ANOVA Table for Apparent Amylose of Rice

Rice Variety	N	Mean	St Dev	SE Mean	P-Value
<i>Jasmine 85</i>	5	15.386	0.260	0.12	0.000
<i>Viwonor</i>	5	18.658	0.208	0.093	

Appendix 10: ANOVA Table for Protein Quantities in Rice and Cowpea Flours (Saline Buffer)

Source	DF	SS	MS	F	P-Value
Sample	5	10095.08	2019.02	260.77	0.000
Error	6	46.45	7.74		
Total	11	10141.54			

Appendix 11: ANOVA Table for Protein Quantities in Rice and Cowpea Flours (Urea Buffer)

Source	DF	SS	MS	F	P-Value
Sample	5	27112	5422	39.07	0.000
Error	6	833	139		
Total	11	27945			

Appendix 12: ANOVA Table for Protein Quantities in Rice and Cowpea flours (DTT Buffer)

Source	DF	SS	MS	F	P-Value
Sample	5	12084.4	2416.9	33.65	0.000
Error	6	430.9	71.8		
Total	11	12515.3			

Appendix 13: ANOVA Table for Protein Quantity of Extrudates with Saline Buffer

Source	DF	SS	MS	F	P-Value
Sample	5	28.37672	5.67534	669.41	0.000
Error	6	0.05087	0.00848		
Total	11	28.42759			

Appendix 14 ANOVA Table for Protein Quantity of Extrudates with Urea Buffer

Source	DF	SS	MS	F	P-Value
Sample	5	402.046	80.409	457.11	0.000
Error	6	1.055	0.176		
Total	11	403.102			

Appendix 15 ANOVA Table for Protein Quantity of Extrudates with DTT Buffer

Source	DF	SS	MS	F	P-Value
Sample	5	350.525	70.105	97.67	0.000
Error	6	4.306	0.718		
Total	11	354.831			

Appendix 16: ANOVA Table for Pepsin Digestion of Rice and Cowpea Proteins

Source	DF	SS	MS	F	P-Value
Sample	5	2.25694	0.45139	141.43	0.000
Error	6	0.01915	0.00319		
Total	11	2.27609			

Appendix 17: ANOVA Table for Pancreatin Digestion of Rice and Cowpea Proteins

Source	DF	SS	MS	F	P-Value
Sample	5	3.491	0.698	4.25	0.019
Error	12	1.972	0.164		
Total	17	5.463			

Appendix 18 ANOVA Table for Pepsin Digestion of Proteins in Extrudates

Source	DF	SS	MS	F	P-Value
Sample	5	1.45540	0.29108	112.68	0.000
Error	6	0.01550	0.00258		
Total	11	1.47090			

Appendix 19 ANOVA Table for Pancreatin Digestion of Proteins in Extrudates

Source	DF	SS	MS	F	P-Value
Sample	5	0.997	0.199	1.46	0.273
Error	12	1.639	0.137		
Total	17	2.636			

Appendix 20: ANOVA Table for Accessible Thiols in Rice Flours

Source	DF	SS	MS	F	P-Value
Sample1	1	0.00000	0.00000	0.00	0.984
Error	2	0.01375	0.00687		
Total	3	0.01375			

Appendix 21: ANOVA Table for Accessible Thiols in Rice and Cowpea Flours

Source	DF	SS	MS	F	P-Value
Sample	5	1.2929	0.2586	18.67	0.001
Error	6	0.0831	0.0139		
Total	11	1.3760			

Appendix 22: ANOVA Table for Accessible Thiols in Extrudates

Source	DF	SS	MS	F	P-Value
Sample	5	402.046	80.409	457.11	0.000
Error	6	1.055	0.176		
Total	11	403.102			

Appendix 23: Consumer Preference Test Questionnaire

Ballot sheet for evaluating the acceptability of extruded breakfast cereal

Name: Date:

Gender:

Age:

Instructions

You have been provided with nine samples of extruded rice-cowpea breakfast cereal. Please evaluate by indicating your degree of liking each of the listed attributes of the samples. Please rinse your mouth before starting. Taste samples from left to right. Please clean your palate or rinse your mouth with water in between samples.

Using the hedonic scale provided, rank the samples in order of preference by indicating your degree of liking for each attribute of each sample. Write the code of the sample in the box next to the liking score that best describes your liking.

1-Dislike Extremely

2--Dislike very much

3-Dislike moderately

4-Dislike slightly

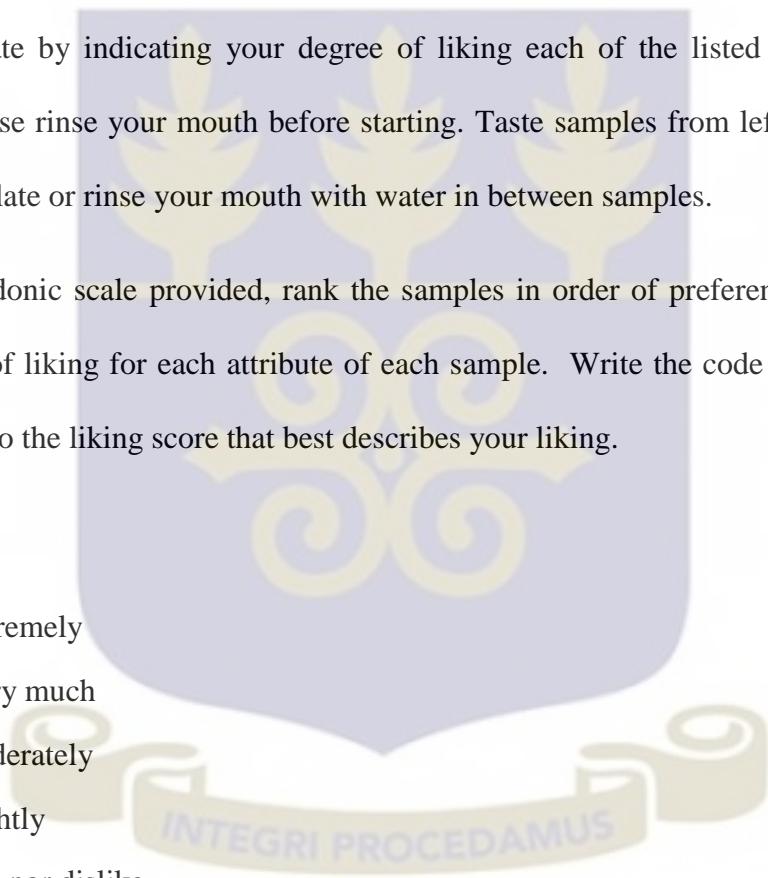
5-Neither like nor dislike

6-Like slightly

7-Like moderately

8-Like very much

9-Like Extremely



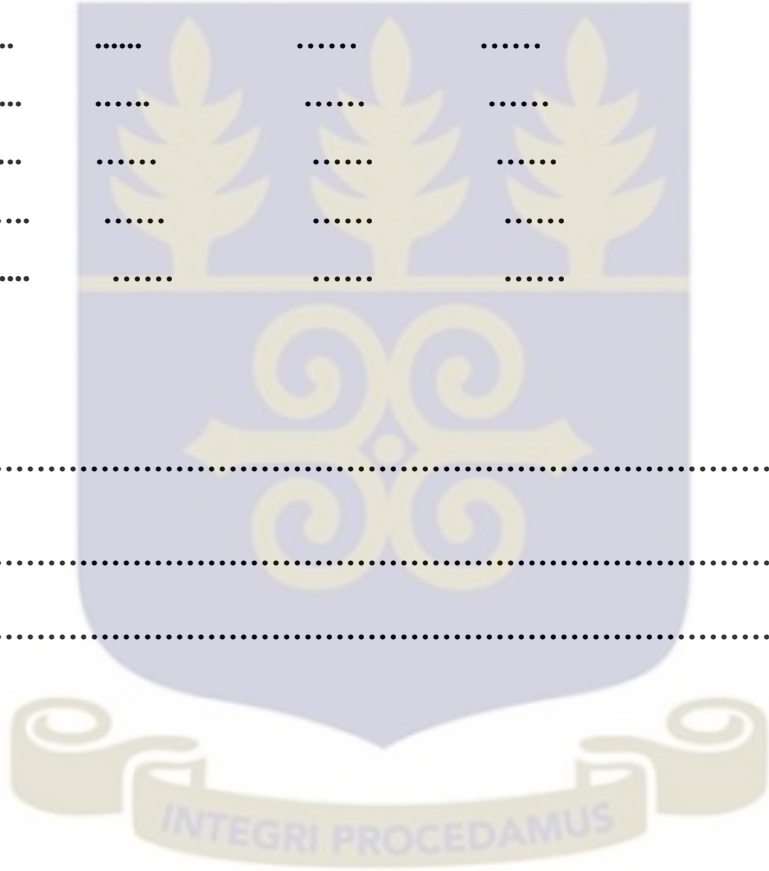
Code	Colour	Aroma	Taste	Crunchiness	Overall Acceptability
<input type="checkbox"/>
<input type="checkbox"/>
<input type="checkbox"/>
<input type="checkbox"/>
<input type="checkbox"/>
<input type="checkbox"/>
<input type="checkbox"/>
<input type="checkbox"/>
<input type="checkbox"/>
<input type="checkbox"/>
<input type="checkbox"/>
<input type="checkbox"/>
<input type="checkbox"/>
<input type="checkbox"/>

Comments

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Appendix 24: Moisture Contents of Raw Samples

Sample	Moisture Content (%)	Average	Std. Deviation	Coefficient of Variation
<i>Viwonor</i>	11.67	11.34	0.47	4.14
<i>Viwonor</i>	11.00			
<i>Jasmine 85</i>	10.29	10.37	0.12	1.16
<i>Jasmine 85</i>	10.46			
Cowpea 0	5.46	5.42	0.05	0.90
Cowpea 0	5.39			
Cowpea 1	8.34	8.40	0.08	0.95
Cowpea 1	8.45			
Cowpea 2	7.21	7.06	0.21	2.97
Cowpea 2	6.92			
Cowpea 3	8.03	8.00	0.04	0.52
Cowpea 3	7.97			



Appendix 25: Moisture Contents of Extrudates

Sample	Moisture Content (%)	Average	Std. Deviation	Coefficient of Variation
Extrudate 1	7.50	7.48	0.03	0.46
Extrudate 1	7.45			
Extrudate 2	8.20	8.08	0.18	2.18
Extrudate 2	7.95			
Extrudate 3	8.69	9.17	0.67	7.35
Extrudate 3	9.65			
Extrudate 4	7.41	7.40	0.08	0.26
Extrudate 4	7.38			
Extrudate 5	7.18	7.28	0.14	1.88
Extrudate 5	7.38			
Extrudate 6	6.31	6.44	0.17	2.67
Extrudate 6	6.56			

Appendix 26: ANOVA Table for Peak Viscosity of Rice and Cowpea Flour Formulations

Source	DF	SS	MS	F	P-Value
Sample	5	348514.0	69702.8	1241.00	0.000
Error	6	337.0	56.2		
Total	11	348851.0			

Appendix 27: ANOVA Table for Final Viscosity of Rice and Cowpea Flour Formulations

Source	DF	SS	MS	F	P-Value
Sample	5	649981.4	129996.3	1495.64	0.000
Error	6	521.5	86.9		
Total	11	650502.9			

Appendix 28: ANOVA Table for Pasting Temperature of Rice and Cowpea Flour Formulations

Source	DF	SS	MS	F	P-Value
Sample	5	115.7701	23.1540	346876.60	0.000
Error	6	0.0004	0.0001		
Total	11	115.7705			

Appendix 29: ANOVA Table for Breakdown Viscosity of Rice and Cowpea Flour Formulations

Source	DF	SS	MS	F	P-Value
Sample	5	141995.7	28399.1	587.57	0.000
Error	6	290.0	48.3		
Total	11	142285.7			

Appendix 30: ANOVA Table of: Total Colour Difference (ΔE^*) of Extrudates

Source	DF	SS	MS	F	P-Value
Sample	5	4.4041	0.8808	10.25	0.007
Error	6	0.5158	0.0860		
Total	11	4.9199			

Appendix 31: ANOVA Table of Hue (h*) of Extrudates

Source	DF	SS	MS	F	P-Value
Sample	5	463.92	92.78	38.56	0.000
Error	6	14.44	2.41		
Total	11	478.36			

Appendix 32: ANOVA Table for Setback Viscosity of Rice and Cowpea Flour Formulations

Source	DF	SS	MS	F-Value	P-Value
Sample	5	232771	46554	108.48	0.000
Error	6	2575	429		
Total	11	235346			

